THE RELATION OF FISHES TO IONS IN THEIR NATURAL ENVIRONMENTS

Reactions and Resistance of Fishes in their Natural Environment to Acidity, Alkalinity and Neutrality.

Reactions and Resistance of Fishes in their Natural Environment to Salts.

BY

MORRIS MILLER WELLS

B. S. University of Chicago, 1912

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

DOCTOR OF PHILOSOPHY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

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REACTIONS AND RESISTANCE OF FISHES IN THEIR NATURAL ENVIRONMENT TO ACIDITY, ALKALINITY AND NEUTRALITY.

MORRIS M. WELLS.

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I. INTRODUCTION.

The present paper is the first of a series that is to deal with the relation of fishes to ions in the natural environments. It is purposed to point out some of the close correlations which exist between the physiology of fishes and their behavior, and to present evidence concerning the importance of such correlations in biological investigation in general. The data presented in the following pages deal with the reactions and resistance of fresh water fishes to acidity, neutrality and alkalinity;

the discussion of the data shows that the phenomena outlined receive much support from the work of other investigators and that the environmental factors which are important to fresh water fishes are probably of importance to many, if not all, other organisms as well.

The investigation has been carried on at the University of Illinois, in Professor V. E. Shelford's laboratory. The work has been done in connection with another line of inquiry regarding the reactions and resistance of fishes to salts. The results of this second investigation will appear as the second paper of the series.

II. APPARATUS AND METHODS.

Two general types of experiments have been run, namely, reaction experiments, and resistance experiments.

A. Reaction Experiments.

This method of experimentation was devised by Shelford and Allee ('13) and may be designated as the "gradient method." In brief the procedure is as follows: A solution gradient is established in an observation tank, the fish introduced, and its movements graphed. The graph, together with notes taken at the time, makes up the experimental record. Fig. 1 shows the type of tank used. A similar tank was used by Shelford and Powers ('15) in their experiments with marine fishes. A black hood screens the tank, the movements of the fishes being viewed through slits in the front of the hood.

The tank has a plate glass front and is lighted by symmetrical lights placed above. A plate-glass cover fits into the top and rests against the surface of the water. This cover is useful in experiments with gaseous gradients as it lessens the vertical gradient due to escape of gas at the surface.

The water flows into the tank through the openings (inlets) in the ends, then toward the middle; at the middle the water from the two ends mixes, the water from each end drifting somewhat past the middle, thus forming the gradient. The water flows out through the exits (outlets) in the bottom and at the top of the tank. An experiment consists of first establishing the gradient, and then introducing the fish and graphing its movements.

In establishing the gradient the flow at each end of the tank was fixed at 500 c.c. per minute in practically all the experiments. The flow of tap water was regulated to 500 c.c. per min. at one

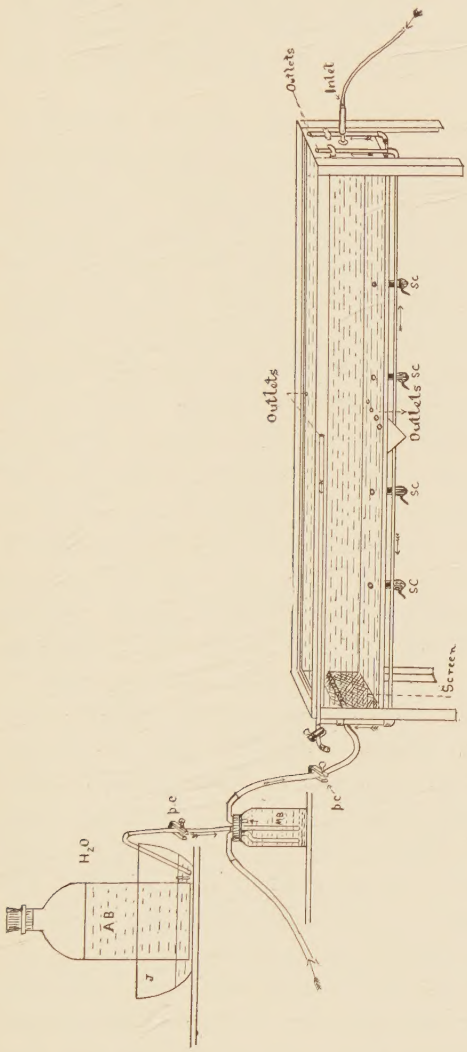

FIG. 1. Showing the gradient tank in which the reaction experiments were performed. The water flowed in at the *inlet* in each end, was distributed by the brass tee (*T*), drifted slowly to the middle, diffusing across somewhat, and out through the *outlets*. One fourth flowed out through each of the two outlets at the top and one eighth through each of the four at the bottom. The experimental factor was introduced from the aspirator bottle (*AB*). This bottle was filled with a solution five times as concentrated as that desired in the experiment. The solution was siphoned out of the jar (*J*) at the rate of 100 c.c. per min. into a mixing bottle (*MB*) into which tap water was flowing at the rate of 400 c.c. per min. This gave a flow into the tank, of 500 c.c. per min., and the desired concentration. Tap water, or water modified to a different degree, flowed into the opposite end at the same rate. Samples for testing were withdrawn through the stopcocks (*SC*) or from above by means of a pipette.

end and to 400 c.c. per min. at the other. Then 100 c.c. per min. of a solution of treated water was introduced into the 400 c.c. flow from a mixing bottle (*M B*). The 100 c.c. flow was kept

constant by using an aspirator bottle as in Fig. 1 (*A B*). This bottle was filled with treated water, corked at the top and placed in the jar (*J*). The water was siphoned from *J* and the pressure was constant as the solution escaped from *A B*, when the level began to fall in *J*. The strength of the solution in the aspirator bottle was always five times that desired in the treated water end of the tank.

The following variations of the simple graphing method of recording were used by Shelford and Allee and have been introduced here. (1) In experiments where the fish was decidedly inactive and remained in one end of the tank, it was driven into the opposite end with a rubber-tipped glass rod. The driving was done at regular intervals and was repeated at similar intervals in the controls. Dotted lines in the graphs indicate that the fish was driven. Experiments of this sort were few in number and have for the most part been thrown out. In some experiments, however, the fishes were active and yet remained constantly in one end of the tank. Driving was again resorted to, in some cases, to make sure that the selection of the given end was a reaction to the gradient. A return to the original end would indicate this to be the case. (2) A number of experiments was performed with 4–10 small fishes in the tank at the same time. These experiments were recorded by readings taken 30 seconds apart. The readings indicate per cent. of fishes in each third of the tank at the time of reading. (3) Usually the fishes were not placed in the tank until the flow at the ends had been going for some time. Thus a gradient was established before the fishes were introduced. In some cases, however, the fishes were either left in the tank when the ends were reversed, or introduced before a gradient had formed. The results of these experiments do not differ from the others except in per cent. of time spent in the thirds of the tank.

The controls were blank experiments, run with untreated water flowing in at both ends, or with no flow at either end. Experiments with the treated water first at one end and then at the other, also served as controls.

The gradient was determined by simple titration with standard acid or alkali, using phenolphthalein or methyl orange

(or both) as indicator. The samples were collected by means of a pipette inserted to a given level below the surface, and the titrations were always made at once, with as much care as seemed necessary. For instance samples containing a high concentration of CO_2 need to be titrated with rapidity, while samples containing H_2SO_4 or KOH may be titrated without haste.

B. Resistance Experiments.

The procedure in the resistance experiments was very simple in most cases. In general the desired solutions were made up from standard solutions of the acid or alkali (measured from burettes) and the fishes introduced. Temperature was controlled by setting the jars containing the solutions, in running tap water. As the experiment proceeded, samples for testing were withdrawn when necessary, and the same amount of water was removed from the control. General controls were kept running throughout the entire time, while numerous temporary controls were set up as demanded by individual experiments.

The species of fishes used principally, have been the blue-gill (*Lepomis pallidus*), white crappie (*Pomoxis annularis*), green spotted sun-fish (*Lepomis cyanellus*), and bull-head (*Ameiurus melas*). Most of the fishes were caught (by seining) in the small streams in the vicinity of the university (the crappie came from a small artificial lake); all were brought into the laboratory with as little handling as possible, and placed at once in large aquaria. They were fed from day to day but fishes do not always eat well in confinement and as time went by they became more or less starved. The changes in the reactions of fishes, which accompany starvation have been investigated and will be discussed in another paper.

The chemicals used have been the chemically pure preparations of Kahlbaum or the analyzed preparations of Baker.

III. THE WATER.

An investigation of the reactions of fishes to salts in solution was begun at Chicago in 1912, at the suggestion of Dr. Shelford. In the fall of 1914 Dr. Shelford left Chicago to take a position at the University of Illinois and the writer accompanied him to

continue the work in his laboratory at that place. The differences in the water supply of the two institutions brought up a number of new questions regarding the reactions of the fishes, and it was decided that to continue the investigation satisfactorily, more must be known of the effects of acid and alkaline water upon fishes and their reactions. This second investigation was therefore taken up and the results are published in advance of those with the salts, since they bear directly upon the interpretation of the latter. A brief comparison of the water of the two institutions will be profitable at this point.

The water at Chicago is pumped from Lake Michigan and analyses show it to be considerably different in gaseous and solid content from the water at Illinois, which comes from deep wells. In 1912 Allee, who had been working at Chicago on rheotaxis in isopods, came to Illinois, bringing with him a stock of animals. In his paper ('13) he compares the waters of the two institutions, and gives a table showing the differences in the dissolved content. This table is inserted here.

TABLE I.

A COMPARISON OF CHEMICAL ANALYSES OF CHICAGO AND UNIVERSITY OF ILLINOIS TAP WATER.

Analysis of solids in parts per million, and gases in cubic centimeters per liter.

	Chicago Tap.	U. of I. Tap.
Potassium, K	6.0	2.6
Sodium, Na	42.1	29.0
Ammonium, NH_4	0.04	2.3
Magnesium, Mg	11.3	34.9
Calcium, Ca	34.6	70.1
Iron, Fe	0.15	1.0
Aluminum, Al	0.00	1.3
Silica, Si	3.3	18.9
Nitrate, NO_3	1.7	0.7
Chlorine, Cl	12.0	3.5
Lead, Pb	0.01	0.00
Sulphuric acid, SO_3	0.04	2.3
Oxygen, O	10.46	0.12
Free carbon dioxide, CO_2	2.5	18.0
Half bound CO_2 (bicarbonates)	32.5	101.12

Allee states that the change of water did not greatly affect the rheotactic response of the isopods. At Illinois he kept the

animals in aërated water, which was thus saturated with oxygen (5.5 to 7 c.c. per liter) while the free carbon dioxide was removed. If aërated sufficiently, the Illinois tap water becomes alkaline to phenolphthalein, and upon writing to Allee with regard to this matter he gives me permission to state that he kept his stock of isopods in such alkaline water for a period of 22 weeks, without increased mortality. He points out that the per cent. of rheotactic response, after a large number of trials, was 8 per cent. less than at Chicago but does not know whether or not to regard this as significant.

Table I. shows that Illinois water contains 18 c.c. of CO_2 per liter and practically no oxygen. Either of these conditions would alone prove fatal to fishes, while the combination would be doubly fatal (Wells, '13). Since aëration removes the CO_2 and at the same time saturates with oxygen, it was thought that this would fit the water for supplying the fish aquaria. The water as it came from the tap was therefore run through the aërating pans which form a part of the apparatus described by Shelford and Allee ('13). The device consists of a series of galvanized pans, placed one beneath the other. The water runs into the upper pan and trickles down through successive pans into a galvanized tank. From the tank, pipes lead to the aquaria. The flow into two large aquaria was regulated to 500 c.c. per minute for each and the fishes were now brought in from the nearby streams and placed in the aquaria in rather large numbers. The aquaria were 8 ft. x 2 ft.; about 300 small fishes were placed in each. This was overcrowding, but fishes have been kept successfully for some time, in closer quarters at Chicago.

The immediate mortality of the stock was not great. It was noted that the darters and other more sensitive fishes did not live well but the sunfishes, bullheads and minnows seemed to be normal. In a few days, however, these fishes began to die. It was thought that the water contained too large an amount of carbonates and an arrangement was made to introduce sulfuric acid into the aërated water at the galvanized supply tank. Enough acid was added to convert about one third of the carbonates into sulfates and with some benefit. It had been noted in the experiments that the fishes did not swim about as actively as

usual and that their sensitivity seemed to be lessened, as they would swim into factors to which they are normally very negative. It was at this point that the study of the effects of acidity, etc., was decided upon.

Tests showed that the water entering the aquaria was practically neutral to phenolphthalein, varying a little from day to day. To determine the effect of the neutral water upon the fishes, a number was taken from the aquaria and placed in tubs of partially aërated tap water (water contained 6–10 c.c. CO_2 per l.). After a day or so in this water, they began to behave normally in the gradient again. The flow of water into the aquaria was now modified by diminishing the amount of aëration. The tap water was run down a wooden trough 12 ft. long, into the aquaria. This saturated it with oxygen but left it decidedly acid with CO_2. From now on the mortality of the stock of fishes was very low. The aquaria were not so crowded as at first, but that the decrease in the number of fishes does not explain the low mortality, will be brought out in experiments to be presented later.

The importance of the chemical reaction of the water to fishes had been foreseen (Wells, '13, p. 337) and it was decided that the peculiar properties of the Illinois water offered an excellent opportunity for continuing this investigation. At the same time it was thought that the work might perhaps throw some light upon a number of the reactions of fishes to salts, which seemed difficult to explain.

The advantages of the Illinois water are due to the following chemical properties: As it flows from the tap it is acid to phenolphthalein from the excess (18 c.c. per liter) of CO_2, and alkaline to methyl orange because it contains a large quantity (101 c.c. per liter) of bicarbonates in solution. The bicarbonates have been formed from carbonates according to the equation $CaCO_3 + H_2CO_3 \leftrightarrows Ca(HCO_3)_2$ and when carbonates are dissolved under the influence of excess of carbonic acid they are practically all converted into bicarbonate, the quantity of unconverted carbonate being negligible (Stieglitz, '09, p. 246, Seyler, '94, p. 105). Under the pressure in the water pipes, there exists an equilibrium between the carbonic acid and the bicarbonates,

but when the water flows out of the tap, the pressure is removed and the carbonic acid at once begins to dissociate into CO_2 and water. The CO_2 passes off into the air and the dissociation of the acid continues until equilibrium with the CO_2 in the atmosphere is established. Parallel with the dissociation of the carbonic acid there goes an increasing tendency for the bicarbonates to break up to form the normal carbonate, and by the time the acidity from the carbonic acid has diminished to approximate neutrality, the bicarbonates are producing a sufficient quantity of the normal carbonate to give the water an alkaline reaction to phenolphthalein. Thus by regulating the amount of aëration, the water can be left acid, made neutral or even alkaline.

Biologists speak of the carbonates, bicarbonates, and carbonic acid, as fixed, half bound and free CO_2, respectively. The fixed is that existing as simple carbonates, the half bound that necessary to convert the carbonates into bicarbonates, and the free that remaining in excess (Seyler, '94, p. 104). It will be seen that the bicarbonates contain both fixed and half bound CO_2, *i. e.*, CO_2 which is to become half bound is added to CO_2, that is already fixed, to form the bicarbonates. Failure to recognize this fact often leads to confusion when these terms are used.

The amounts of the three kinds of CO_2 can be determined accurately by titration, using two indicators, phenolphthalein and methyl orange. Methyl orange is unaffected by H_2CO_3 and hence the bases present as carbonates or bicarbonates can at once be titrated with acid. Carbonates are alkaline to phenolphthalein, bicarbonates are neutral, and free CO_2 is acid. A carbonate titrated with acid, therefore, becomes neutral to phenolphthalein (if titrated under conditions which prevent loss of CO_2) when the carbonates have all been converted into bicarbonates.

Methyl orange is not affected by H_2CO_3 because this acid does not produce a high enough concentration of H ion. The indicator is however very sensitive to OH ion and reacts to the minute amounts that are present in a bicarbonate solution. Phenolphthalein, on the other hand, is very sensitive to H ion

but not to OH ion. It therefore gives an acid reaction with CO_2 but is unaffected by the minute amount of OH ion which is present in solutions of bicarbonates.[1] Methyl orange will give an alkaline reaction in water in which the concentration of H ion is considerably greater than that of OH ion. Thus, in the presence of CO_2, titration with this indicator is not a determination of true alkalinity for the water is as a matter of fact acid, since it contains a higher concentration of H than OH ions. Marsh ('07) makes this error when he states (p. 337) that "the reaction of water which will support fish life must be slightly alkaline." His determinations were made with sulfuric acid, using methyl orange as indicator. The water to which he refers ("Potomac service water") was in all probability acid to phenolphthalein. Marsh also states that "when the water becomes even slightly acid, fishes cannot live in it." This would mean that fishes can not live in water which has been made slightly acid to methyl orange by the addition of an acid. I have added sulfuric acid to tap water until it gave an acid reaction to methyl orange, and find that fishes live in it as well as in the original tap water, *i. e.*, normally. The fishes should not be placed in such water until some little time after the adding of the sulfuric acid, however, for in the process of changing the carbonates to sulfates, a large amount of carbonic acid is liberated ($CaCO_3 + H_2SO_4 \leftrightarrows CaSO_4 + H_2CO_3$) and until this carbonic acid has dissociated and the CO_2 passed off into the atmosphere to a large degree, its presence will kill fishes which may be introduced. The amount of carbonic acid formed will depend upon the amount of carbonates in the water. (The reaction with bicarbonates will give the same result.)

In the following pages I shall introduce experimental data to show that fresh water fishes cannot live normally in water that is alkaline but that they require a certain degree of acidity to carry on their normal activities.

[1] Noyes ('13) gives a table (p. 388) showing the acidity or alkalinity of solutions at the change of color for various indicators. Methyl orange gives an alkaline reaction when the OH concentration is only 10^{-9} while phenolphthalein is not affected until the concentration of OH ion reaches 10^{-5}. Methyl orange gives an acid reaction when the H ion concentration is 10^{-4} while phenolphthalein reacts when the H ion concentration is only a little more than 10^{-8}.

IV. PRESENTATION OF DATA.

The following experiments show the effect of different degrees of acidity and alkalinity, upon the reactions and longevity of fresh water fishes.

A. Reactions of Fishes to Acidity and Alkalinity.

1. *Reaction to Acids.*

(*a*) *To Carbonic Acid.*—A number of experiments was run to determine the reactions of the fishes to this acid. Three degrees of acidity were used for the most part: (1) Neutral, to very faintly acid (aërated water) (2) moderately acid (8–10 c.c. CO_2 per liter; obtained by using half and half mixture of 1 and 3); (3) strongly acid water (unaërated tap; 18 c.c. CO_2 per liter).

(1) *Moderately Acid Water vs. Strongly Acid Water* (*Graph 1, Chart I.*).—The fishes selected the lower acidity with much precision. They also spent much time at the surface, as is characteristic when the concentration of CO_2 is high.

(2) *Slightly Acid vs. Moderately Acid Water* (*Graph 2, Chart I.*). —The fishes were left in the tank, and the flow altered so that the moderately acid water ran into the end that had previously been strongly acid and neutral water was run into the opposite end. The fishes were graphed after five minutes. They definitely selected the end of the tank into which the neutral water was flowing. Test showed this end to contain 3 c.c. CO_2 per liter. Seven experiments with this combination were run and all gave similar results. Variations were due to specific and size differences. The larger fishes and the crappies and green spotted sunfishes selected a somewhat higher acidity than the smaller fishes, especially the blue-gills.

(3) *6 c.c. CO_2 per Liter vs. 4 c.c. per Liter* (*Graph 3, Chart I*).—The concentrations of CO_2 were obtained by regulating the amounts of aërated and unaërated water. Six experiments were run. The bullheads and blue-gills selected the lower concentration with precision, while the sunfishes and crappies chose the higher end with as much definiteness. Thus, as was seen in (2), the species differ in the optimum CO_2 concentration which they select at this time of year.[1] The difference in

[1] Because of the fact that the resistance of fishes varies with the season (Wells, '14), it is very probable that the CO_2 concentration selected by a given species will show seasonal variations also. This point is yet to be investigated.

specific reaction may be in part a matter of size, as the crappies and sunfishes averaged larger than the blue-gills. Small sunfishes were, however, found to be less sensitive to CO_2 than were blue-gills of the same size. In this case the reaction is correlated with resistance as the sunfishes are more resistant than the blue-gills. The bullheads, however, are perhaps the most resistant of our fresh water fishes yet they are very sensitive to CO_2. The sensitiveness of the bullheads is probably related to the peculiarity of their integument which (Herrick, '02) has chemical perceptors "taste buds" scattered over its entire surface.

So far the fishes had for the most part selected the end of the tank containing the largest proportion of neutral water, *i. e.*, the lowest acidity. To ascertain definitely the reactions to the neutral water, the following experiments were performed.

(4) *Slightly Acid (3 c.c. CO_2 per Liter) vs. Neutral Water.*)—The slightly acid water was obtained by partially aërating the water which flowed into one end. This was done by running it through a galvanized tank which was a part of another piece of apparatus. The gradient tested practical neutrality at one end, and slight acidity at the other. The fishes definitely selected the end containing the CO_2 and were thus negative to the neutral water.

(*b*) *Reactions to Sulfuric Acid.*—It should be pointed out that experiments where other acids than H_2CO_3 are added to water, which contains bicarbonates, are open to misinterpretation if an attempt is made to compare the reactions of the animals to the acids, in this way. The addition of a strong mineral acid to such water does not result in the presence of a hydrogen ion concentration from the mineral acid itself, until all the bicarbonates have been decomposed (*i. e.*, changed to sulfates, etc.) or, in other words, not until the water has become acid to methyl orange. The reaction is a double decomposition, $H_2SO_4 + Ca(HCO_3)_2 \leftrightarrows CaSO_4 + 2H_2CO_3$. Until this reaction is completed, the chief result of adding the mineral acid will be to increase temporarily the concentration of carbonic acid by liberating the fixed and half bound CO_2. The concentration of H ion from this weakly ionized acid will be but a small per cent. of that which would have been obtained from the min-

eral acid itself; yet this will be the only hydrogen ion supply until the bicarbonates have all been changed to sulfate (if sulfuric acid is the mineral acid used). The tap water at Illinois requires approximately 90 c.c. of .1 *N* H_2SO_4 to neutralize the bicarbonates in one liter of water while the Chicago water requires about a third as much. According to the above equation one molecule of the acid liberates 2 molecules of CO_2. Therefore 90 c.c. of .1 *N* acid will liberate 210 c.c. of CO_2. Powers ('13) did not take this reaction into account and speaks of comparing the reactions of crayfishes in gradients of HCl and CO_2. As a matter of fact the amounts of HCl which he added to the Chicago tap water were probably used up in neutralizing the bicarbonates. Thus all his gradients were with carbonic acid, and the differences which he gets are due to the liberation of this acid in excess, in the reaction of the HCl and the bicarbonates.

(1) *H_2SO_4 to Neutralize all Bicarbonates vs. Neutral Water.*—The CO_2 liberated in this gradient made the water so acid that the fishes were soon overcome, and died if not removed from the tank. However at first they gave a decidedly negative reaction to the acid end.

(2) *H_2SO_4 to Liberate 40 c.c. CO_2 per Liter vs. Neutral Water.*—The fishes reacted much as they did in gradients of aërated vs. unaërated water. They were very negative to the acid end.

(3) *H_2SO_4 to Liberate 4 c.c. CO_2 per Liter vs. Neutral Water (Graphs 4 and 5, Chart I.).*—Eighteen experiments of this sort were run. Of the 18 graphs, 14 show that the fishes spent 90 per cent. of the time in the acid half of the tank; 3 show more than 50 per cent. of the time in this half and 1 (small blue-gill) shows an 80 per cent. preference for the neutral end. That the fishes are negative to neutral water is thus confirmed. To ascertain the chemical reaction of the water at the point in the tank where the fishes turned back from the neutral end, numerous samples were titrated from this region, during the experiment. They showed that the water at this point contained about 1 c.c. of CO_2 per liter. The graphs shown in Chart 1. are typical for all.

CHART I.

Showing the reactions of the fishes to different degrees of acidity. The gradient is between the two kinds of water, indicated at the top of each graph. Numbers at the left of the chart indicate time in minutes. Strongly acid = 18 c.c. CO_2 per liter; moderately acid = 8–10 c.c. per liter; slightly acid = 2–3 c.c. per liter; and neutral water = actual neutrality to 1 c.c. per liter. Dotted lines indicate that fish was driven.

(*c*) *Reaction to Acidity in Distilled Water.*—The distilled water, which was available in quantity from the chemistry department, was not rapidly toxic to the fishes and since the foregoing results are of some general biological importance, it was decided to repeat the experiments in distilled water. This water was faintly acid with CO_2 containing 2–3 c.c. per liter. It contained no salts; so the addition of a strong acid resulted in no complications such as those discussed in the case of the tap water. A number of experiments was performed with various strengths of acid and alkali. The neutral portion of the tank was kept track of by means of titrations and the reactions of the fishes to this neutral region es-

pecially noted. The results are presented in Table II.; in brief they are as follows. The fishes spent practically all the time in the acid portion of the tank, turning back from the alkaline end at a point just on the acid side of neutrality. They did not, however, select the highest acidity available, but swam back and forth in the tank between neutrality on the one hand and about .0002*N* H_2SO_4 on the other. The small amount of CO_2 present in the distilled water may be neglected in the presence of the much more ionized acid. At the range of dilution used in these experiments, carbonic acid would have to be about 1,400 times as concentrated as sulfuric acid, to give an equal concentration of H ion.

TABLE II.

SHOWING THE REACTIONS OF FISHES TO ACIDITY AND ALKALINITY IN DISTILLED WATER.

Acid Used.	Concentration.	Reaction.
H_2CO_3	.00004 *N*	Negative: choose higher acidity.
H_2CO_3	.0001 *N*	Positive: some fishes choose this concentration in preference to either higher or lower acidity.
H_2SO_4	.0005 *N*	Very negative.
H_2SO_4	.0002 *N*	Still very negative.
H_2SO_4	.00005 *N*	Positive when neutral water is the other choice.

The fishes used did not select alkaline water in any case except when the only other choice was neutrality. Then they spent most of the time on the alkaline side, rather than at the neutral point.

2. *Reactions to Alkalies.*

(*a*) *Alkalies in Neutral Water.* (1) *Na_2CO_3 (.01 N) in Neutral Water vs. Neutral Water.*—Six experiments were run with this combination. The results were rather indefinite. However, the graphs as a whole show a slight preference for the alkaline half of the tank. As has been pointed out already, the fishes are negative to the neutral water, and these experiments confirm this reaction, even though the only other choice is alkalinity.

(*b*) *Alkalies in Strongly Acid Water.*—In this water which is acid with CO_2 (18 c.c. per liter), the first action of the alkali will be to neutralize the acid. Thus a small amount of alkali introduced at one end will simply produce an acid gradient by

lessening the acidity at this end. Eighteen c.c. of CO_2 equals an .0008 *N* solution. In most cases, the concentrations of alkali used have been much greater than this and the amount used up in neutralizing the acid may be looked upon as negligible. In some experiments, to be cited, the acid factor is of much importance.

(1) *Na_2CO_3 (.01 N) in Strongly Acid Water vs. Strongly Acid Water (Graph 1, Chart II.)*.—The fishes stayed in the middle of the tank, coming to the surface very little. The gradient was acid at one end and alkaline at the other. Titrations showed that the fishes spent most of the time on the acid side of neutrality.

(2) *Na_2CO_3 (.002 N) in Strongly Acid Water vs. Strongly Acid Water (Graph 2, Chart II.)*. Fifteen experiments were run with this combination. The graphs show that the fishes spent most of the time nearer the alkaline end than before, but titration showed that they were merely following the neutral point, remaining on the acid side most of the time.

(3) *Na_2CO_3 (.0005N) in Strongly Acid Water vs. Strongly Acid Water (Graph 3, Chart II.)*. This concentration of alkali was just a little more than enough to neutralize the acid in the water of the alkaline end. The end was really slightly acid, however, from the diffusion of more acid from the acid end of the gradient. The fishes moved into the so-called alkaline (really slightly acid) end and remained there during the experiment. This was true for all the fishes used.

(4) *$NaHCO_3$ (.01N) in Strongly Acid Water vs. Strongly Acid Water.*—This salt is neutral to phenolphthalein as has been pointed out in the preceding discussion. A number of experiments, recorded both by graphs and readings at short intervals, were run with it. The results were not at all definite. The fishes seemed to be indifferent to this bicarbonate in acid water, or else they were not at all stimulated by its presence.

(5) *NH_4OH in Moderately Acid Water (Made it Faintly Alkaline) vs. Moderately Acid Water (Graphs 4 and 5, Chart II.)*. Ten experiments with this alkali were run, to check up Shelford and Allee's work ('13) with it. They say (p. 252) that the fishes (*Abramis*) did not react to ammonia in a concentration which caused them to turn on their sides after an hour or more. In

my experiments, I found also that the fishes do not react to this alkali with the precision found for the other alkalies used.

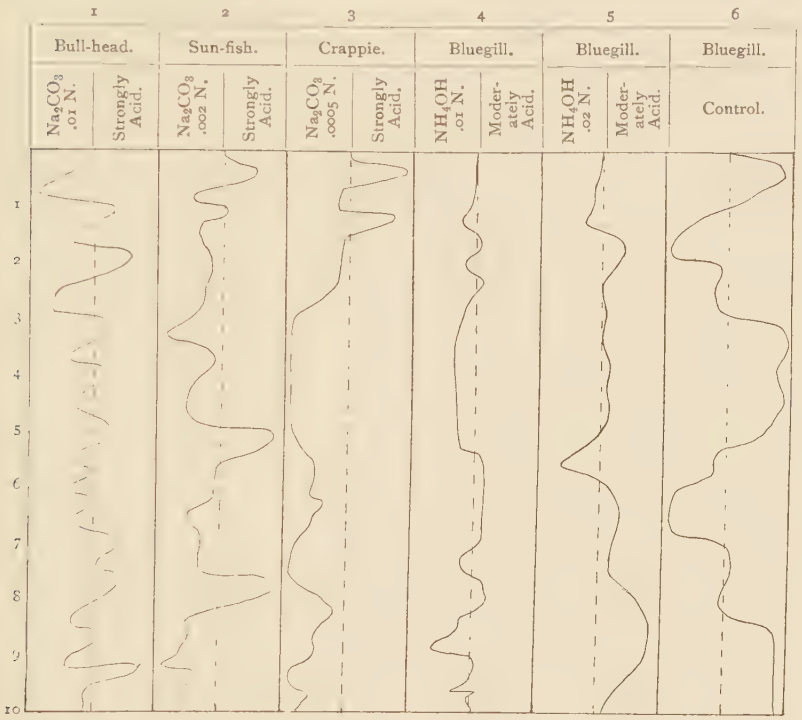

CHART II.

Showing the reactions of the fishes to alkalies. The gradient was between the two kinds of water indicated at the top of each graph. Strongly acid water = 18 c.c. CO_2 per liter; and moderately acid = 8–10 c.c. per liter. Numbers to left = time in minutes.

In the first experiments a .005 *N* solution was run into one end of the tank. The fishes selected the middle of the tank for the most part, though one blue-gill was positive to the ammonia end. The concentration of OH ion was of course very low, with so small a concentration of so weakly ionized a base, and since other experiments have shown that blue-gills are less negative to neutrality than are other fishes, this reaction is not surprising. The ammonia concentration was raised to .01 *N* and the fishes, blue-gill included, moved toward the tap-water end of the tank. Later the concentration of the alkali was

raised to .02 *N*, but even now the avoidance of the alkali end was not nearly so definite as in the experiments with the other alkalies. Graphs 4 and 5 (Chart II.) show this indefinite reaction very clearly. In the .02 *N* gradient, the fishes were soon overcome by the toxicity of the water, which they selected, and they died there if not removed.

The fact that fishes fail to recognize ammonia in solution is of considerable importance, for this substance is being introduced into fish waters in many kinds of sewage. Furthermore it will be shown in the second paper of this series, that the gas has not lost its toxicity even when it has been converted into its various salts. The chemical explanation of the failure of the fishes to recognize and react to the presence of fatal concentrations of the hydrate in solution is probably due to the fact that the concentration of ammonia as gas, reaches a fatal concentration before the concentration of OH ion stimulates the fishes sufficiently to cause them to react negatively. They do not appear to react to the gas itself. Noyes ('13, pp. 203–4) states that ammonia dissolves in water, in part, without chemical change and that it is probable that a large part of the ammonia exists, as such, in the solution. He quotes Moore ('07) as calculating that only 30–40 per cent. of the ammonia exists as ammonium hydroxide, NH_4OH, at 20° C. Noyes thinks that the per cent. may be even less than this.

Again, the solution of ammonia diffuses through the water with great rapidity; much more rapidly than do most other substances. To determine the rate of diffusion, a little phenolphthalein was added to the aspirator bottle (*AB*, Fig. 1) containing the ammonia solution. The pink solution could be seen as it moved through the tank, and in less than a minute it had spread over the entire surface, and to a lesser extent, had penetrated the deeper water. Because of this rapid diffusion, no perfect gradient could be established with this substance. It may also be noted that ammonia behaves just opposite from the salts, the latter spreading along the bottom. In the ammonia experiments, the fishes seldom approach the surface, while in strong carbon dioxide gradients, they spend much time gulping the surface film. Shelford and Allee ('13, p. 231) state

that in open tanks the amount of CO_2 at the surface is markedly less than at deeper levels.

3. *Conclusions Drawn from the Reaction Experiments.*

The reaction experiments recorded in the previous pages suggest the following conclusions. (1) Fresh-water fishes are negative to neutrality in favor of either slight acidity or slight alkalinity. Their normal choice is slight acidity (about .00005 *N* H_2SO_4 or .0001 *N* CO_2). (2) Species of fishes differ in the degree of acidity selected. Blue-gills select water that is but very slightly acid (1–2 c.c. CO_2 per liter, *i. e.*, .0001 *N* carbonic acid) while crappies select a concentration of from 4–6 c.c. CO_2 per liter. (3) The principal stimuli to which fishes react are H and OH ions. They do not react to ammonia, as a gas in solution.

B. Resistance Experiments.

It has been pointed out that the stock of fishes did not live well in the aquaria when these were supplied with water, which was neutral, or nearly so; to determine more exactly the reasons for the high mortality, between 50 and 60 experiments were performed. Some of these experiments lasted through a number of weeks, while others were finished in a few hours. The fishes were placed in different concentrations of acid and alkali in partly aërated water (from the aquaria) and in distilled water.

1. *Resistance to Acids.*

The resistance of fishes to carbonic acid has been worked out (Wells, '13) and it was decided to try the effects of other acids. Ten experiments with sulfuric acid in distilled water are summarized in Table III. The table shows that there is a concentra-

Table III.

Showing the Resistance of Fishes (3 Gram Blue-gills) to H_2SO_4 in Distilled Water.

Concentration of Acid.	Dying Time in Hours.
.001 *N*	3.5
.0005 *N*	7.0
.0002 *N*	42.0
.00015 *N*	60.0
.000075 *N*	Alive and vigorous at end of a month.

tion of this acid in distilled water, at which the fishes in question live as well as though in tap water. Higher concentrations of acid are fatal, the time required to kill the fishes being proportional to the hydrogen ion concentration.

2. *Resistance to Alkalies.*

In a .001 *N* solution of KOH in distilled water, a 3 gram blue-gill lived 4 hrs. and 25 min. In a .0005 *N* solution, a fish of the same size was alive at the end of 10 days. Titration at this time showed that the water had become acid to phenolphthalein from the CO_2 given off in the metabolism of the fish. The experiment was discontinued. To make sure that the fish in the first experiment had not been killed by the toxic potassium ion, another 3 gram blue-gill was placed in a .01 *N* solution of $NaHCO_3$ in distilled water. At the beginning, this solution was neutral, but it was expected that the bicarbonate would dissociate and the solution would become slightly alkaline from the carbonate thus formed. A blank control, containing the same amount of bicarbonate, but no fish, was run. The fish in the experiment died on the third day. Titration showed that the water had become .0009 *N* alkaline. The control was .001 *N* alkaline. Blue-gills therefore do not live well in water which is even very slightly alkaline.

3. *Resistance to Neutrality.*

The foregoing experiments, together with many facts recorded in the literature, suggested the possibility that the fact that it is neutral may have something to do with the toxicity of distilled water. Thirteen experiments were performed to test this possibility in a preliminary way. The facilities available did not make it possible to experiment with absolutely neutral water, but the results obtained are suggestive, as neutrality was approached very closely in some cases. Most of the experiments were performed with water that came from a copper still and will be referred to as once-distilled water. A few experiments were performed with a much purer water which was the once-distilled water redistilled in a better still and coming in contact with little copper. In neither kind of water could the amount

of copper have been especially large, however, for small blue-gills lived in both kinds as well as in tap-water, so long as the water was slightly acid.

A comparison of the conductivities of the two kinds of water showed that the once-distilled had a conductivity[1] of 600 $\times$ 10^{-7} while the conductivity of the twice-distilled was only 10 $\times$ 10^{-7}. These conductivities are for 25° C. The conductivity of the water probably does not indicate the amount of copper present however for the metal is in all likelihood present in the colloidal state. Mengarini and Scala ('12) have shown that a number of metals, including copper, form a colloidal solution with distilled water even at room temperature, and especially in the absence of air. The conditions in a still would be especially favorable for the reaction, since the temperature is high and air excluded.

The addition of an acid to a colloidal solution would tend to precipitate the colloid, and this undoubtedly explains in part the effect of addition of acid in making distilled water less toxic,[2] as it will be shown that it does. Since, however, it has been shown (Bullot, '04) that distilled water which contains no copper is still toxic to organisms, other factors must be concerned. The evidence of the experiments presented in the present paper, indicates that the neutrality of the water is one of these factors.

It has been suggested in the preceding pages that the blue-gills and crappies differ in respect to the hydrogen ion concentration which they select and their resistance to the distilled water bears out this point as the crappies die in it in a day or so, while the blue-gills live indefinitely.

(1) *Experiments with Once-distilled Water.*—This water was slightly acid to phenolphthalein and was neutral to methyl orange. Its toxicity was tested by placing fishes in jars containing a liter of the water. A 12-gram crappie died in this

[1] The conductivity of pure water is 1 $\times$ 10^{-7}.

[2] Locke ('95) calls attention to the fact that poisonous distilled water may lose its poisonous properties (if due to copper) by long boiling, and especially when brought into contact with sulphur, carbon, manganic oxide, cotton wool, silk, and other substances. The effect is very probably again due to the precipitation of the colloidal copper.

water in 2 days, but when this same liter of water was divided into two parts and a 3-gm. blue-gill placed in each part, both fishes were normal at the end of a month. In Table IV. is given a summary of a number of experiments performed with blue-gills in once-distilled water.

TABLE IV.

SHOWING THE RESISTANCE OF SMALL BLUE-GILLS (3–5 GRAMS) TO DISTILLED WATER THAT IS BARELY ACID WITH CO_2.

Conditions of Expt. Fish Placed in,	Resistance of Fishes.
1. Freshly distilled water	Normal after 5 days; expt. discont.
2. Boiled distilled water	Normal after 5 days; expt. discont.
3. Distilled water plus Na_2CO_3 to make neutral	Normal after 2 days. Water acid again.
Added a little $NaHCO_3$ to (3) to keep neutral	Dead on 10th day.
4. In dist. water as in (1)	Normal after 30 days.

Table IV. shows that the once-distilled water is not greatly if at all toxic to the blue-gills, but experiment 3 shows that these fishes cannot live in the water if it is slightly alkaline. This same distilled water is rapidly toxic to the crappies and sunfishes, however, as was shown in an experiment already described and in those which follow. This lack of resistance of the sunfishes in particular is a complete reversal of the ordinary specific resistance of these fishes as compared with the blue-gills, for in carbon monoxide, ethylene, sulphur dioxide, etc., the sunfishes are much more resistant than are the blue-gills.

On January 30, a liter of water (once-distilled) was made .00005 *N* acid with H_2SO_4 and another liter left as it came from the still. An 8-gram crappie was placed in each jar. The fish in the pure distilled water was dead in 12 hrs. while the one in the distilled water made acid, lived for 65 hrs. Several other experiments of this sort gave similar results, showing that the crappies cannot live in the neutral distilled water when it is pure, as well as they can when it is made slightly acid. It is very probable that sightly higher concentrations of acid than those used would have prolonged the lives of these fishes even more successfully than the .00005 *N* but as the stock of fishes was running low, these experiments were reserved for another time.

An experiment with small bullheads is very interesting. Normally the bullheads are perhaps the most resistant fresh-water fishes known. In the reaction experiments they selected a rather low concentration of hydrogen ion but were decidedly on the acid side of neutrality. In the pure distilled water a bullhead (4 in. long) lived 8 days; another in distilled water made .00005 *N* acid, lived for 20 days.

(2) *Resistance to Doubly Distilled Water.*—This water was less toxic to the crappies than was the once distilled water, as it contained less colloidal copper. It has been pointed out that the toxicity of the once-distilled water was lessened by the addition of acid, partly because the acid precipitated the colloidal copper. The experiments indicate further, however, that the neutrality of the water must be reckoned with also. This is again brought out, and more definitely, by a few experiments with the twice-distilled water. A quantity of this water was placed in a large bottle and a solution of barium hydroxid was suspended over it. At the end of a week the water was practically neutral. Two portions were taken in 500 c.c. Erlenmeyer flasks and a small bullhead (2.5 in.) placed in each. One portion was left neutral and the other made slightly acid with H_2SO_4. The fish in the neutral water lived 16 days and the one in the acid water 19 days. A few other experiments were performed with the twice-distilled water and all gave similar results. The stock of fishes was about exhausted, however, and further experiments were delayed until another time.

V. GENERAL DISCUSSION.

The fact that in natural bodies of water the chemical reaction of the water may vary from alkalinity through neutrality to acidity or the reverse, makes the practical importance of a knowledge of the reactions and resistance of fishes and other organisms to such chemical conditions an obvious one. From the experiments and discussion which have gone before, it is clear that water which gives an alkaline reaction to phenolphthalein for any length of time during the year, is undesirable as a home for most fresh-water fishes. On the other hand, marine fishes (Shelford and Powers, '15) with the exception of the anadro-

mous species, probably would not survive in water which was even faintly acid. Since algæ and other phytoplankton forms (Birge and Juday, '11 and '12) may cause a body of water to become partially or wholly alkaline, through their ability to dissociate the bicarbonates, vegetation in fish waters assumes a line of importance heretofore little considered. The effects of sewage upon the acidity or alkalinity of natural bodies of water must also be reconsidered in the light of its possible injurious or beneficial effects due to its chemical reaction. Thus a large number of interesting and important questions suggest themselves.

The effect of the chemical reaction of the water upon the distribution of organisms promises much room for investigation. There is no doubt but that fishes recognize the difference between very faintly acid or very faintly alkaline, and neutral water. Henderson's work ('13), upon the mechanism which maintains a constant proportion of H and OH ions in the blood of animals, suggests the physiological reason for this extreme sensitiveness of the fishes. It is clear that even very small variations in the proportions of these two ions in the blood of the organism, are of grave importance, and we find in the blood a combination of gases and salts that makes such variations impossible as long as the animal is normal. The blood will maintain its normal chemical reaction (just on the alkaline side of neutrality) in the face of relatively large changes in the environment, yet we know that the mechanism breaks down when the change is either too great or too long continued (acclimatization is not considered at this time). The hyper-sensitiveness of the animals to the chemical reaction of the water, in the case of aquatic organisms, is another important factor in preserving the normal reaction of the blood, as the reactions of the organisms work in a way that causes them to turn back from concentrations of H or OH ion that would be detrimental. The delicacy and accuracy of these reactions are evidenced in the reaction experiments which have been discussed in the preceding pages.

The physiological effect of the acid, neutral, and alkaline water upon the organism very probably has to do with decrease or increase in the permeability of the exposed tissue cells (es-

pecially gills in case of fishes). Osterhout ('14) has shown that in plant cells alkalies increase the permeability up to death; acids however at first produce a rapid decrease in permeability, followed later by an increase which continues up to death. The concentrations of acid used by Osterhout were .001 *N* to .03 *N*. Very low concentrations such as those used in the experiments discussed here would very likely maintain a permanent decrease in the permeability of the cells, and the concentrations of acid in which the fresh-water fishes normally live, may thus protect the fishes by decreasing the permeability of their gills and preserving the normal reaction of the blood. Alkaline water, on the other hand, does not do this for fresh-water fishes, and they soon succumb in it. The results of Shelford and Powers ('15) indicate that the action of alkaline water upon marine fishes is to produce a normal permeability of the membranes and it may be that acid water would kill these fishes by decreasing the permeability below normal.

The effect of neutrality upon the permeability of tissues has not been worked out, so far as I am aware, but since fresh-water fishes, and probably marine fishes also, are negative to neutral water, it must be that such water exerts a marked effect upon the permeability, or some other physiological condition, in the gill membranes. The negativeness of organisms to neutral water indicates that they are either over-stimulated in such water, or under-stimulation sets up internal disturbances. Thus they may avoid it because of its non-stimulating character. It may well be that in neutral water, the normal chemical reactions do not go on, for acidity and alkalinity surpass all other conditions, even temperature and concentration of reacting substance, in their influence upon many chemical processes. Of all the catalytic agents, H and OH ions are by far the most important, and in their influence upon the stability of colloidal systems they are unapproached by other substances (Henderson, '13).

Birge and Juday ('11 and '12) attempt to explain the vertical distribution of the plankton in the lakes of Wisconsin and New York, upon the basis of relation to oxygen and food. This attempt has, it seems to me, met with little success, and they

themselves point out many contradictions. According to their idea, the plankton forms must in many instances be reacting positively to concentrations of oxygen which are as small as .1 c.c. per liter, or even less. This supposition is contrary to all the experimental evidence regarding the reactions of aërobic fresh-water organisms to this gas. In an attempt to correlate the distribution of the zoöplankton with the chemical reaction of the water, I have gone over Birge and Juday's tables and figures, and have come to the conclusion that such correlation exists. Their data indicate in practically all of the lakes (in the summer condition) a point at some depth below the surface of the lake, where the organisms are more numerous than at any other depth. In many cases this rise is proportionately very high and is usually of small amplitude. Thus the large number of forms occurs in a rather limited region vertically. After the rise, there is a marked diminution in the number of forms and then again at a little greater depth there is another increase, smaller than the first, but still very noticeable in their curves. This increase is followed by a second diminution. The first diminution usually occurs in or near the thermocline where the temperature often shows a very sudden lowering. The oxygen supply sometimes falls off here also, but not always, and in the lakes to which I refer particularly, the oxygen supply is practically the same at all depths. A very important fact, however, is that the water in the region of the thermocline, *i. e.*, at the region of smallest numbers of plankton, is often neutral or very nearly so (summer condition). Above this region the water is alkaline, and below acid. From the data given in Birge and Juday's Tables XVIII. and XIX. ('12, pp. 602–608), I have compiled the following table (Table IV.) to show the relation of the zoöplankton to this neutral region. Birge and Juday's Table XVIII. is a record of temperatures and gas contents at the different depths; Table XIX. is an analysis of the plankton catches made in ten lakes. The records for a given lake were all made on the same day. Table XVIII. gives titration records which show that in three of the lakes at a definite depth, the water was neutral. Table XIX. gives the plankton collections at different depths in these three lakes, on the same

day. Table IV. inserted below, is made up from a combination of the data found in the two tables; most of the data in Table IV. refer to the three lakes in question. In the instance of *Triarthra*, however, the data come from two other lakes, as this form does not occur in the three lakes from which the other data are taken.

Table IV.

Showing the Relation of Zoöplankton to Neutrality.

The table is compiled from Birge and Juday's ('12) Tables XVIII and XIX, Most of the data are taken from their records for the three lakes, Canandaigua, Seneca, and Skaneateles. In these lakes the neutral depth was accurately located by titration of samples. The titrations and plankton collections were made on the same say. The data for the rotifer triarthra are taken from lakes Hemlock and Keuka as these are the only lakes in which this form occurs in the records. n.c. = no collection at this degree of acidity or alkalinity. The figures in the columns indicate the number of forms per cubic meter of water.

Name of Animal.	Alkalinity in C.c. per Liter of CO_2 to Make Neutral.			Neutrality.	Acidity in C.c. of CO_2 per Liter.		
	3–2.	1.5–1.	.5–.25.	0.	.25–5.	.75–1.	.1–1.5.
Pleosoma (R)	3,925	0	0	0	0	0	0
Vorticella (P)	12,250	0	0	0	0	0	0
Asplanchna (R)	11,320	400	0	0	0	0	0
Dinobryon (P)	43,700	19,130	42,800	0	0	0	0
Diaphanosoma (C)	2,885	2,750	n.c.	260	0	0	0
Nauplei	28,150	28,050	13,250	570	140	40	205
Diaptomus (Co)	7,850	6,660	17,350	2,220	1.440	390	100
Conochilus (R)	130	290	250	250	30	65	30
Anuroea (P)	4,000	1,250	200	30	20	20	20
Cyclops (Co)	13,775	7,620	7,620	25	30	0	5
Notholca (R)	625	685	65	0	65	0	0
Daphnia (C)	1,260	650	400	130	1,145	25	0
Ceratium (P)	52,330	104,500	85,160	2,025	11,760	5,750	1,670
Polyarthra (R)	12,350	1,620	2,350	160	1,190	1,240	40
Malamonas (P)	0	n.c.	770,400	95,600	1,900	0	1,900
Triarthra (R)	0	n.c.	0	n.c.	1,050	1,110	2,425

Letters in parenthesis after name of animal indicate the following. (R) = Rotifer; (P) = Protozoan; (C) = Cladoceran; (Co) = Copepod.

Table IV. shows (1) that all the zoöplankton forms are more numerous on either the acid or the alkaline side of neutrality, than they are at neutrality itself, *i. e., they are negative to neutrality;* (2) some forms as *Pleosoma* and *Vorticella*, are found only in the alkaline water; (3) others range between slight alkalinity and slight acidity but are never very numerous at neutrality and often (*Daphnia, Ceratium*, etc.) show an increase on either side; (4) a few forms (*Triarthra*) occur wholly on the acid side of neutrality.

The factors that regulate the distribution of the plankton in the lakes are undoubtedly numerous. The only certain way to determine them is to investigate experimentally the reactions of the animals to the factors concerned, both singly and in combination. To do this would be tedious but not especially difficult. As an index to the distribution of these forms, I believe that the presence and position of a neutral layer of water will be found to be important.

Besides the experimental data presented in the papers by Birge and Juday, the literature contains much other experimental evidence which bears directly upon the question of the toxicity of neutrality to organisms. Much of this evidence is found in connection with experiments upon the toxic effects of distilled water, and the action of salts in antagonizing this toxicity. In a series of papers published by Ringer and his students between the years 1883 and 1893 the question of the toxicity of distilled water was investigated and its reality apparently demonstrated. It was also shown that various salts are effective in neutralizing this toxicity, some being much more efficacious than others. In 1893 Naegeli showed that for Algæ (*Spirogyra*) at least, the toxicity of distilled water was due to contamination from the copper stills in which it was prepared. Locke ('95) confirmed Naegeli's results by showing the effect upon certain fresh-water animals to be due also to the minute amounts of copper present, and Ringer ('97) again taking up the subject reversed his former conclusions and confirmed those of Locke. Jennings ('97) found that *Paramœcia* live for weeks in distilled water. Moore ('00) says that young trout and tadpoles (unfed) live as long in distilled as in tap water, *i. e.*, several weeks. Lillie ('00) says that *Planaria maculata* will live in distilled water. Pure distilled water seemed then not to be toxic to fresh-water animals though apparently toxic to most marine animals. *Fundulus* eggs seem to be an exception among marine animals (Loeb, '99), as they can live in distilled water for weeks and still produce normal embryos. In 1903 Bullot after testing the effects of distilled water upon the fresh-water amphipod, *Gammarus* concluded that pure distilled water was toxic to this crustacean. Bullot's experiments were performed with great

care; he considered and seemed to have eliminated the following possible toxic factors: copper, impurities from the glass, low oxygen, ammonia, and carbon dioxide. He found also that NaCl in small concentrations would neutralize the toxicity of the pure water to such an extent that the animals lived almost as well in an .00008 *N* solution of this salt as in the natural fresh water. The toxicity of pure distilled water, he concluded, is due to the lack of salts in solution. Peters in 1908 performed some very careful experiments to test the effect of pure distilled water upon protozoa. He came to the conclusion that distilled water which contains no salts, and which is changed often enough to prevent their accumulation from the metabolism of the animals, is rapidly toxic to these forms.

I have gone over the above papers and have found many statements which indicate that the presence of a certain concentration of hydrogen ion, was beneficial to the animals experimented upon. For instance, Ringer ('83) states that the distilled water which he used killed minnows, on an average, in 4.5 hours. He also says that the distilled water was very faintly acid; so faint was the acidity that he did not rely upon his own judgment but had others make the test also. However, he says, to prove that the acidity was not the cause of the death of the minnows, he took three liters of water and to one added 6 drops of 10 per cent. acetic acid, to the next 12 drops and to the third 20 drops. He then placed three minnows in each liter of acid solution. After 24 hours, the minnows were "quite natural" and he concluded, therefore, that the acidity could not have been harmful in the case of the distilled water. This conclusion of Ringer's illustrates the attitude taken by most authors with regard to the presence of acid in the water, that is, the acid is looked upon as a detrimental factor, to be considered negatively. So far as I have been able to read, the authors quoted above have taken little consideration of the possibility that the presence of a certain concentration of H or OH ion is essential to the welfare of animals.

This Ringer does not suggest, even though the minnows in the acid water were well on the way to live as long as any of the animals kept in salt solutions. In this same paper, Ringer notes

that when he put a large number of fishes (up to a maximum) into a given volume of distilled water, they lived longer than one or two fishes placed in the same volume of water. He attributes this to the excretion by the fishes of inorganic salts, and does not take into consideration the carbon dioxide factor which would have increased the acidity of the water to many times that of the almost neutral distilled water. Again, in speaking of the salts which are best for preserving life in distilled water, Ringer states that the calcium salts are better than those of sodium and potassium, that $CaSo_4$ is better than $CaCl_2$ and that the phosphate of lime $(Ca_3PO_4)_2$ is much superior to all the other salts. This latter salt, he states, is decidedly acid, and he says ('86) "it is interesting to observe that though the circulating fluid with phosphate of lime gives a slight acid reaction to delicate blue litmus paper it will sustain contractility of muscle for hours." Thus a small hydrogen ion concentration seems to be beneficial, if not essential, to the continued life and activity of the organisms and tissues in question.

The question of the existence of a carbon dioxide optimum for animals has received considerable investigation with varying results. Ringer in 1893 investigated the influence of carbonic acid upon the frog's heart and concluded that free CO_2 in saline solution arrests cardiac contractility. He does not state what concentrations of CO_2 were used, but since he speaks of passing carbonic acid through the solution "for some time," his solutions were probably very acid. In a few experiments he neutralized the slightly acid distilled water which was used to make up the saline solutions, with NaOH, and noted that in this neutral solution, the contractions of the heart very soon became abnormal. Jerusalem and Starling ('10) review the literature regarding the importance of carbon dioxide for the ordinary functions of the body, and report a series of experiments to determine its influence upon the beat of the heart of the frog, tortoise and mammal (cat). They conclude that the CO_2 tension in the blood must be maintained at a certain height, if the pumping action of the heart is to be normally carried out. In their review of the literature they point out that their conclusions are in accord with those of Miescher, Haldane, Mosso, Hender-

son, and Bottazzi (see pp. 279–280). The lowest concentration which Jerusalem and Starling used was 2 per cent. of an atmosphere or about 20 c.c. CO_2 per liter. Their highest ran up to 200 c.c. per liter. Hooker ('12) tested the effect of carbon dioxide upon muscular tone and, in opposition to Jerusalem and Starling, concluded that this gas does not appear to be directly beneficial to tissues, except in case of intestinal muscle rhythm. He thinks it may be indirectly beneficial. Like most other workers upon this problem, Hooker used very high concentrations of the gas. His concentrations varied from 5 per cent. to 20 per cent. of the gas, in the atmosphere to which the solution bathing the tissue was exposed. Water will dissolve nearly its own volume of CO_2 and thus the concentration of carbonic acid varied from 50 to 200 c.c. of CO_2 per liter. The smallest concentration used would kill most fresh-water fishes in a short time.

Reuss ('10) worked upon the effect of CO_2 upon the respiration of fishes and concluded that it is an important one. The regulation according to him is through the respiratory center and not peripheral as Bethe ('03) believed. Shelford and Allee ('13*a*) note the extreme sensitiveness of fishes to CO_2 in gradients, and think the production of the gas as a product of the metabolism of the organism may tend to increase its external effect when the fishes come in contact with water containing it.

Bullot ('04) in his work with the fresh-water amphipod (*Gammarus*) noted, as did Ringer in the case of fishes, that the animals lived longer in distilled water when a number was present in a given volume, or in other words, when the volume of water per individual was small. He says: "If the amount of water falls below a certain limit, the animals will live the longer, the smaller the amount of water, provided the quantity does not fall below a certain minimum." In Table V. I have collected Bullot's data showing this point. The table shows that the relation holds for *both redistilled water and water distilled in copper alone.* The length of life in the water from the copper still is proportionately shorter throughout.

TABLE V.

SHOWING THE RESISTANCE OF AMPHIPODS IN DISTILLED WATER, WHEN EQUAL NUMBERS OF ANIMALS ARE PLACED IN DIFFERENT VOLUMES OF WATER, OR WHEN DIFFERENT NUMBERS OF ANIMALS ARE PLACED IN EQUAL VOLUMES OF WATER (COMPILED FROM BULLOT, '04, PP. 204–5).

Per Cent. of Animals Alive After 2 Days.

No. Animals.	Volume of Water.	Water.	
		Redistilled in Glass.	Distilled in Copper.
1	Same throughout	45%	Proportionately less throughout.
5		80%	
10		90%	

Time Required to Kill One Half the Animals.

Same throughout	5 c.c.	10 days	8 days
	20 c.c.	2.5 days	1.5 days
	50 c.c.	1.5 days	1 day
	100 c.c.	same	same.

In considering the possible importance of CO_2 as a factor in the toxicity of distilled water, Bullot states that the water which he used was very faintly acid to phenolphthalein, but not enough to injure the animals. He says: "We know from the works of P. Bert that, for cold-blooded animals like the frog, for instance, CO_2 is toxic only when its concentration in the air reaches 15 per cent. This corresponds to a solution of 15 per cent. of this gas by volume, as the water dissolves its own volume of CO_2 at ordinary temperature and normal pressure. This quantity is 350 times larger than the one which could be found in the distilled water." A 15 per cent. solution of CO_2 means 150 c.c. per liter of water. 1/350 of this is .42 c.c. In other words the distilled water used by Bullot was practically neutral, since the amount of hydrogen ion to be obtained from so small a quantity of so little ionized an acid as carbonic acid, would be almost negligible. In the gradient experiments cited in this paper, I have shown that certain fishes are negative to so small a concentration of CO_2 as 1 c.c. per liter, in preference for slightly higher concentrations. I have further shown that these fishes do not live as well in distilled water that is practically neutral, as they do in the same water made slightly (.00005 *N*) acid. Thus the existence of a hydrogen ion concentration optimum for these forms seems to be clearly demonstrated.

Peters ('08) makes no mention of the possibility of the neutrality of the distilled water which he used, having something to do with its toxicity, yet in a previous paper ('07) he recognizes the impor ance of the presence of a certain concentration of hydrogen ion for the existence of certain protozoa in hay infusions. On page 346, he says: "The data obtained indicate that, of the chemical conditions, the concentration of acid . . . is one of the chief factors determining the biological content and history of a culture."

From the data and discussion that have gone before, it seems certain that the chemical reaction of the water is a factor of marked importance in the life history of fresh-water animals. Some fresh-water forms are apparently positive to alkalinity as seen in the fresh-water lakes (Birge and Juday, *loc. cit.*) and others, that normally live in water that is acid with CO_2 are not killed by living in alkaline water (isopods). On the other hand, many forms, and probably most of the fresh-water fishes belong here, are always found in acid water if such be available, and these forms cannot live normally in neutral to alkaline water. Shelford and Powers ('15) have shown that marine fishes select the alkaline side of neutrality in a gradient, and in this difference in the behavior of the fishes, lies a key to the fundamental physiological difference in the organisms of these two habitats. Fresh-water fishes must live in the presence of an excess of hydrogen ion if their life processes are to be carried on in normal fashion. Shelford ('14) states that the carbon dioxide content of the water over the breeding grounds of fresh-water fishes should not average more than 1 c.c. per liter, nor exceed 5 c.c. during the summer months. This statement is probably wrong in limiting the average to 1 c.c. per liter for some fishes, as the green spotted sunfish and the crappies are negative to this small concentration of CO_2 showing a preference for slightly higher concentrations. Blue-gills, on the other hand, select a degree of acidity that is very little above neutrality. The CO_2 concentration selected by fishes probably varies with the season, and certainly with the salt content of the water in which they live. The variations of the CO_2 optimum in salt concentration will be discussed in the second paper of the series.

One thing is clear; the chemical reaction of the water should be known with accuracy, in all experiments with salts and gases in solution. A recognition of this fact will help to clear up some of the many contradictory results which have been obtained by various workers. It seems to be demonstrated beyond a doubt that the toxicity of distilled water is in part due to the absence or scarcity of inorganic salts, but it is also evident that the neutrality of such water may be an important factor in its toxicity.

VI. GENERAL CONCLUSIONS.

1. The chemical reaction of the water is an important factor in the reactions and resistance of organisms.

2. Fresh-water fishes select slight acidity in a gradient, when the other choices are neutrality and alkalinity. They choose slight alkalinity in preference to neutrality.

3. The CO_2 optimum for the different species of fishes experimented upon, varies from very close to neutrality for the blue-gill, to 6 c.c. per liter for the sunfishes and crappies. This is for the November to January condition. At other seasons and in other waters, the optimum probably varies somewhat. The optimum acid concentration for fresh-water fishes in distilled water is about .00005 N H_2SO_4.

4. The distribution of the plankton in the lakes of Wisconsin and New York (Birge and Juday, '11 and '12) shows a very interesting correlation with the chemical reaction of the water. There are fewer animals at neutrality than in the slightly alkaline and slightly acid water just above and below the neutral layer. Thus the forms are negative to neutrality.

5. The neutrality of distilled water is a factor to be considered in its toxicity.

VII. ACKNOWLEDGMENTS AND BIBLIOGRAPHY.

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THE REACTIONS AND RESISTANCE OF FISHES IN THEIR NATURAL ENVIRONMENT TO SALTS

MORRIS M. WELLS

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I. INTRODUCTION

In a previous paper (Wells '15 a) the reactions and resistance of fresh water fishes to alkalinity, acidity and neutrality were discussed upon the basis of experimental evidence which seemed to indicate that the chemical reaction of the water (i.e., acid, neutral or alkaline) in which the fishes live, is a matter of considerable importance to fresh water fishes and probably to marine fishes also (Shelford and Powers '15). In the present paper a large number of experiments bearing upon the reactions and resistance of fresh water fishes to salts is presented. Practically no previous work has been published upon the *reactions* of fishes to salts and the main part of the data presented here has to do with this phase of the subject. Some interesting relations between acidity and resistance to salts are also presented. This latter phase of the subject has been worked out in a preliminary way only; the more definite relations are left for further investigation.

The present investigation was begun at the suggestion of Prof. V. E. Shelford and was carried on at the University of Chicago during the years 1912 and 1913. In the fall of 1914 operations were transferred to the University of Illinois as the author accompanied Dr. Shelford in his transfer to that place.

II. THE WATER

The differences in the water of the two institutions have been discussed in the first paper of the series (Wells, l. c.). The chief differences are the following: The water at Chicago comes from Lake Michigan; as it flows from the tap in the laboratory, it is slightly acid with carbon dioxide (2–3 cc. per liter), is supersaturated with O_2 (8–10 cc. per liter), contains 32 cc. per liter of half-bound CO_2 (bicarbonates) and a proportionate amount of other salts. The water at the University of Illinois comes from deep wells. As it flows from the tap it is strongly acid (18 cc. CO_2 per liter), contains practically no O_2 (0.12 cc. per liter) and the half-bound CO_2 equals 101 cc. per liter; other salts are in proportion. Aeration brings the two waters to more nearly

the same condition and fishes can live in either after the proper amount of aeration. Too much aeration causes the Illinois water to become alkaline to phenolphthalein and fresh water fishes cannot live in such water.

III. METHODS AND APPARATUS

The reaction experiments have been performed in the gradient tank used in the acid gradient experiments (Wells '15 a, fig. 1, p. 223).

The salts used have been, the chlorides, nitrates and sulphates, of ammonium, potassium, sodium, calcium and magnesium. In presenting the results of the reaction experiments the salts will be grouped with reference to the anion, as the similarities in behavior, in the different salt gradients, make this a rather natural division. They will also be taken up in the order of increasing toxicity of this ion as worked out by Lillie ('10) and others. Thus the order of consideration will be, chlorides, nitrates, sulphates. In considering the resistance experiments, on the other hand, the salts will be grouped according to the kation. In the gradient experiments the concentration of salt introduced at the salt end has been in nearly all cases 0.01N. In a few experiments the concentration was made 0.02N or even higher in an attempt to drive the fishes out of the salt end, to which they were giving a positive reaction. These experiments will be cited as they come up.

The gradient in the salt experiments was obtained as follows: Tap water was set to flowing into one end of the tank at the rate of 500 cc. per minute, and into the other end at the rate of 400 cc. per minute. A 0.05 N solution of the salt was made up with tap water and run into the flow at the 400 cc. end at the rate of 100 cc. per minute. This made the volume of the flow at the two ends equal. The salt solution was mixed with the tap water, in a mixing bottle, outside the experimental tank. From the mixing bottle a single outlet led to the experimental tank. At first the gradients were tested before and after each experiment. Later, after a very careful study of the gradient

had been made at Chicago, by determining the conductivity of the water at various points in the tank, tests were no longer made. Thus the actual concentrations existing throughout the tank have not been determined in each experiment but the study that was made indicated very clearly that under the given conditions this concentration is almost constant for a given salt. Thus there always exists a gradient of the dissolved salt, between the two ends of the tank. The presence of this gradient is shown by the reactions of the fishes as well as by the conductivities and titrations. That the gradient is not perfect is to be expected; its peculiarities were brought out in the study which

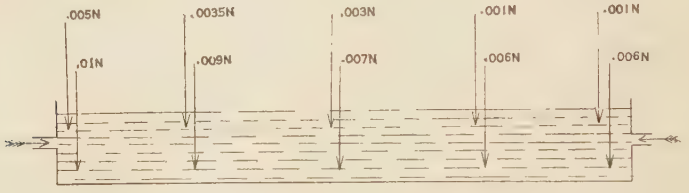

Fig. 1 Longitudinal section through the gradient tank. The figures indicate the concentrations of the salt at the depths indicated by the arrows. These concentrations were ascertained by determining the conductivity of samples taken from the different parts of the tank; in determining the gradient 7 samples along any given level were taken; only five are shown in the figure.

was made by means of the conductivity method. Figure 1 shows the gradient as it existed after the flows at the ends had been running for some time.

It will be noted from figure 1 that at any given level there is a gradient of salt from end to end of the tank. The concentration at the bottom of the tank was much higher than that near the surface of the water, and thus the fishes at times reacted to the vertical gradient, which was much sharper than the horizontal one. This reaction to the vertical gradient did not interfere greatly with the experiments, however, because the fishes tend to swim back and forth in the tank at whatever level they may be. Furthermore, most of the fishes worked with, remained near the bottom for a large proportion of the time. A further,

point brought out by the conductivity measurements was that the water, after flowing in at the ends of the tank for 15 minutes or less, often showed a piling up of the salt at a point about two-thirds of the way to the tap water end, i.e., a little past the middle. This piling up was brought out graphically by the use of colored salt solutions, which showed a more intense color at this point for a short time. Later the deepening in color disappeared, and tests showed the gradient to be continuous from one end of the tank to the other.

Before the fact of the piling up of the salt was discovered, it was noted that the fishes often gave a negative reaction to this part of the tank. With the demonstration of the increased salt concentration at the point in question, and the fact that the increase disappeared after the flow at the ends had been on for about 30 minutes, most of the experiments were delayed until sufficient time had elapsed for the adjustment to take place; any marked reaction of the fishes at the point of higher concentration, was noted and recorded. That the gradient as shown in figure 1 is a typical gradient is supported by the fact that Shelford and Powers ('15) figure a similar gradient which they obtained between sea-water and fresh water, in their work with marine fishes. In the following gradient experiments, attention should be called to the fact that the reactions whether positive or negative are seldom 100 per cent reactions. In other words, the fishes are nearly always positive to some concentration of the salt in question. It seems that for most fresh water fishes there exists an optimum salt concentration somewhere between a 0.01N and that of the tap water. This fact is brought out in the experiments with a majority of the salts.

The species of fishes used principally have been the black bullhead (Ameiurus melas), blue gills (Lepomis pallidus), rock bass (Amblopites rupestris), green spotted sun fish (Lepomis cyanellus), white crappie (Pomoxis annularis), pumpkin seed (Eupomotus gibbosus), and small mouth black bass (Micropterus dolomieu). Numerous experiments have also been run with various species of Cyprinid minnows.

IV. PRESENTATION OF DATA

A. REACTION EXPERIMENTS

1. Reaction to chlorides

The fishes used are less sensitive to the chlorides of the salts than they are to the nitrates and sulphates. They also react differently in the presence of different chlorides. Thus they are sensitive to both the anions and the kations, and to different degrees.

a. Ammonium chloride. The fishes were decidedly negative to this salt in 0.01N concentration. The experiments were run in water that was a mixture of half aerated and half unaerated tap water (i.e., moderately acid with CO_2). It has been found (Wells '15 a) that fishes give normal reactions in this water.

b. Potassium chloride. These experiments were also performed in water which was somewhat acid. The reaction of the fishes was rather peculiar in that they were positive to a higher concentration of this salt than was expected. Twenty-one experiments were performed and all showed this phenomenon. In a number of cases the fishes selected the highest concentration for a large part of the time. It was thought that the reaction might be due to the positiveness of the fishes for the chlorine ion, as will come out in other experiments; the known toxicity of the potassium ion, however, made this conclusion seem doubtful. Again, the fishes had been in the laboratory for over a month and were somewhat starved. It had already been determined that starvation increases the positiveness of some fishes to certain salts, and thus the reaction might be laid to this. However, the real explanation was later found to lie in a mutual antagonism which exists between certain salts and acids. Thus the reaction of the fishes in selecting the salt end was a reaction which brought them into the lesser stimulating part of the gradient. In the tap water end, the CO_2 made the water quite acid. In the salt end this action of the acid was neutralized by the presence of the salt and vice versa. This phenomenon was noted in a number of the gradient experiments, while its cause was definitely proved in the resistance experiments.

c. Sodium chloride. This was the first salt to be experimented with at the University of Illinois and a large number of experiments (46 in all) was performed with it, as the reactions of the fishes were not what was at first expected. Experiments were run in aerated (neutral) water, in moderately acid water (8–10 cc. per liter) and in strongly acid water (18 cc. CO_2 per liter).

It had been noted that the fishes became sluggish when kept in the aerated water, and because they reacted positively to the NaCl in the gradients in this neutral water, the experiments were repeated in acid water to make the results certain. The fishes were positive to the NaCl half of the tank in all three kinds of water, but were markedly most positive in the most acid water. They are negative to this water alone, because of its marked acidity. The increase in positiveness to the NaCl in the acid water must be due to the fact that the salt antagonizes the stimulating action of the acid and thus the fishes selected the portion of the tank where they were the least stimulated, as they did in the case of the KCl gradient in acid water.

In an attempt to drive the fishes out of the salt end, the NaCl concentration was increased to 0.02N but without diminishing the positive reaction. In the strongly acid water the fishes were found to give a positive reaction to as small a concentration of NaCl as 0.001N though the reaction to this low concentration was not so definite as with the higher concentrations. The reaction to NaCl varied somewhat with the species; the crappies and bull-heads were positive in all three kinds of water while the blue-gills were positive in the neutral and strongly acid water but were indifferent to negative, in the moderately acid water.

Ten experiments with 0.01N NaCl, in distilled water, were run to check those with the tap water. The results show the fishes to be markedly positive to the NaCl in distilled water gradients; this positiveness is not as great as in the acid water, but is great enough to show conclusively that the fishes used are positive to NaCl in concentrations very little lower than 0.01N.

d. Calcium chloride. Calcium chloride was the first salt used at Chicago in the gradient experiments. It was found that normal fishes (large rock bass are exceptions) are negative to

a 0.01N solution of this salt, and the graphs show this negativeness to be rather definite. The fishes turned back from the $CaCl_2$ end at a point which the conductivity measurements showed to be about 0.0065N. Some of the apparently normal fishes, however, gave positive reactions to 0.01N $CaCl_2$ and in working out this point over 150 experiments were performed. A very interesting relation between starvation and the reaction of fishes to $CaCl_2$, and probably some other salts, was found to exist. The experiments showing this relation will be discussed on a subsequent page under the heading, "Physiological states and the reactions of fishes" (p. 260).

e. Magnesium chloride. Normal fishes reacted negatively to a 0.01N concentration of this salt, but as with calcium, there was a number of instances where the reaction seemed to be reversed. Normal fishes were also negative to a 0.02N concentration, which however did not prevent a few of the fishes from showing a positive reaction, as they had done with the 0.01N solution.

2. Reaction to the nitrates

The nitrate experiments, with the exception of part of those with calcium, were performed at Illinois. The experiments with the nitrate of calcium were performed largely at Chicago, enough being repeated at Illinois to correlate the reaction in the two waters.

a. Ammonium nitrate. Practically all the fishes used were negative to this nitrate, which is very stimulating to them, in tap water, as will be shown in the resistance experiments. They did not, however, avoid the salt end with as much precision as is displayed in the case of a number of the other salts, and in one experiment, a 25-gram crappie, although giving a fairly strong negative graph, still was overcome by the salt, lost control of its movements, and 'scooted' about the tank, finally leaping over the edge onto the water table. Sixteen experiments were performed; of these fourteen show decidedly negative reactions, while two, one with a 3-gram blue-gill and one with a 6-inch bull-head, show positive reactions. These two fishes were not

overcome by the salt, though they remained in the salt end during a majority of the 15 minutes that they were in the tank.

b. Potassium nitrate. The fishes were consistently negative to this salt in 0.01N concentration. Of 40 fishes tried in the gradient, 27 gave decidedly negative reactions, 5 stayed in the middle third of the tank, and 7 were more or less positive. In only 3 experiments was the time spent in the salt half of the tank, over 60 per cent of the total time. Of the 27 negative fishes, 20 spent over 80 per cent of the time in the tap water end.

c. Sodium nitrate. Experiments with all three kinds of water were run. In the neutral water the fishes were decidedly negative the graphs showing that 86 per cent of the time was spent in the tap water end of the tank. In the moderately acid water, 70 per cent of the reactions were negative and 30 per cent positive. In the strongly acid water, the fishes were decidedly positive to the 0.01N concentration showing an 81 per cent positive reaction. The concentration of the salt was now decreased to 0.002N and the same fishes tried. They were not so positive to this small concentration in the acid water as they had been to the 0.01N solution but they were still more positive than in the moderately acid water. The graphs show 45 per cent of the time was spent in the salt third of the tank, 30 per cent in the middle third, and 25 per cent in the tap water third. These results show again the effect upon the behavior of the fishes, of the antagonistic reaction between the acid and the salt; they select the higher concentration of salt in the gradient in strongly acid water but are negative to this same concentration in water which is not so acid. Note also (table 1, p. 256) that the antagonism between the salts and the acid seems to be more marked in the case of the K salts. Table 1 shows that in the case of both the chloride and nitrate of potassium the antagonism between the salt and the acid was sufficient to cause the fishes to react positively in moderately acid water. With sodium, the chloride shows a positive reaction in the moderately acid water but in this same water, the nitrate gives a negative reaction. It is not until the water has been made strongly acid that the fishes react positively to the nitrate of sodium.

d. Calcium nitrate. At Chicago 20 (40-min.) experiments were run with this salt. The reactions of the fishes were so decidedly negative that further work seemed unnecessary. At the University of Illinois, it was decided to repeat the experiments with calcium nitrate as a check upon the reactions of the fishes in the two waters. To this end a series of experiments with 0.01N $Ca(NO_3)_2$ in neutral water, was run. The results were very different from those obtained at Chicago. There the fishes had shown a 90 per cent negative reaction to this salt in 0.01N concentration, while at Illinois in the neutral water, the reaction was 50 per cent negative and 50 per cent positive. In other words they seemed to be indifferent to the salt. It was thought that the explanation of the Illinois reaction might lie in the fact that, since calcium nitrate hydrolizes to give a faintly acid solution, the fishes, which (Wells '15 a) had already been shown to be negative to the neutral water, were reacting to this acidity. This proved to be the case, for when the experiments were repeated in moderately acid water, the fishes gave an 80 per cent negative reaction.

To make doubly sure of the results with the calcium nitrate, a final series of experiments was run in distilled water, which it will be remembered is slightly acid with CO_2 (2–3 cc. per liter). Five 15-minute graphing experiments were run with results that show a 75 per cent negative reaction. An experiment with 4 bull-heads (3–5 in. long) was read 50 times at 30-second intervals. Computation showed that the fishes had spent 74 per cent of the time in the negative half of the tank. Thus the reactions at Chicago and at Illinois, when slightly acid water is used, are in close agreement in showing the negative reaction of fishes to 0.01N concentration of calcium nitrate.

e. Magnesium nitrate. Twelve experiments were performed with this salt at Chicago; they showed a 100 per cent preference for the tap water half of the tank. The negativeness was more marked in some experiments than in others but in none did the fishes swim into the salt end. The experiments have not been repeated at Illinois.

3. Reaction to sulphates

a. Ammonium sulphate. Fishes are negative to this salt as to the other ammonium salts. All the ammonium salts are strongly toxic to the fishes used. Especially is this toxic reaction noticeable in the tap water. The explanation for this will be taken up in the discussion of the resistance of fishes to ammonium salts.

b. Potassium sulphate. Fishes are decidedly negative to 0.01N concentration of potassium sulphate. Twelve experiments were performed in moderately acid water and in none did the fishes give a positive reaction. In one the fish selected the middle third of the tank, but turned back regularly from the salt end. The results with this salt illustrate the increasing toxicity of the anion; it will be remembered that in moderately acid water the fishes gave a positive reaction to the nitrate of potassium. No experiments were performed at Chicago with this salt.

c. Sodium sulphate. No experiments were performed at Chicago with this salt. At Illinois two series were run, one in moderately acid water, and the other in strongly acid water. The reactions in the two kinds of water were very similar to those obtained with sodium nitrate in the same kinds of water. It was noted that in the strongly acid water the fishes often spent much of the time at the surface and were thus not swimming in the strongest gradient. For this reason the reactions might be expected to be somewhat less definite but the results show very little difference in cases where the fishes stayed at the bottom or swam at the surface. In 4 experiments the fishes spent practically all the time at the surface and 60 to 90 per cent in the salt half of the tank. In 8 experiments with this strongly acid water the fishes remained at the bottom throughout; seven of these experiments show a decided preference for the salt end while one was negative.

In the $NaNO_3$ experiments, it will be remembered, the fishes were negative to the salt in moderately acid water and this was also found to be the case with the sulphate. Fifteen experi-

ments with 0.01N Na_2SO_4 in moderately acid water were run. All of them show decidedly negative reactions. In a number of experiments the attempt was made to drive the fishes into the salt end, but with no success, except in one case, where a 20-gram crappie was driven into the salt end and remained there for 5 minutes before swimming back into the tap water end. The blue-gills could not be driven as they would dart back past the driving rod in every case. If these fishes were dropped into the salt end they showed much disturbance and very soon swam into the tap water end. In one experiment 8 small blue-gills (2–4 grams) were placed in the tank and readings of their position taken every 30 seconds until 25 readings had been made. The percentage of time spent in the thirds of the tank were, salt third 13 per cent, middle third 36 per cent and tap water third 51 per cent. Thus fishes are negative to 0.01N sodium sulphate in moderately acid tap water but are positive to this concentration in strongly acid water. The explanation of this latter reaction must lie in the antagonism between the salt and the acid.

d. Calcium sulphate. These experiments (11 in all) were performed at the University of Illinois. The reaction in moderately acid water was negative in all but two cases. In one of these a fish which had at first selected the tap water end, was driven into the salt end, where it remained for the remainder of the experiment. An experiment in which 10 small blue-gills (2–4 grams) were placed in the tank and their positions read at 30–second intervals, showed percentages as follows: Time spent in salt third 25 per cent, in middle third 32 per cent and in the tap water third 43 per cent.

e. Magnesium sulphate. Ten experiments with this salt were run at Chicago. All showed a negative reaction to the 0.01N concentration and in most cases the reaction was very decided (usually above 80 per cent in tap end). The experiments were not repeated at Illinois.

4. Conclusions from reaction experiments

We note from the data which have been given that fishes are markedly sensitive to salts in solution and that they react to them in a definite manner. They are negative to 0.01N concentrations of most of the salts used, if in water which is moderately acid; this is the normal condition in most natural bodies of water. When the water becomes strongly acid, the reactions of the fishes are modified and may be reversed by the mutual antagonism which exists between salts and acids. So far as these experiments show, this antagonism exists only between the salts of K and Na and carbonic acid. From the general work upon the antagonism of salts, to be discussed later, one would not expect the antagonism to extend to the salts of Ca and Mg. In the reaction experiments it was seen that the fishes are, in nearly all cases, positive to some concentration of the salt in question. This positiveness is most noticeable in the case of NaCl. Table 1 is introduced to summarize the reactions of the fishes to 0.01N salt concentrations in the different kinds of water used.

B. ANTAGONIZING SALTS AND THE REACTIONS OF FISHES

To determine whether or not fishes detect and react to combinations of salts in gradients, a number of experiments was performed, based upon the phenomena of the antagonistic reaction of salts, which are familiar to all biologists. These phenomena in their simplest form are expressed in the antagonism which exists between the salts of Na and K, on the one hand, and Ca and Mg, on the other. There has also been found a certain degree of antagonism between Ca and Mg in some cases (Meltzer and Auer '08). Most of the work on the antagonism of salts has been done either upon single organs as the heart, or other muscle tissue, or upon developing eggs and embryos. Certain combinations have also been shown to be best for preserving the life of fishes and other fresh water animals in distilled water (Ringer, etc.). So far as I am aware, no attempt has ever been made to determine the reactions of fishes to com-

TABLE 1

Showing the reactions of fishes to .01N concentrations of various salts, in gradients of different kinds of water, i.e., waters of different degrees of acidity

SALT	PER CENT POSITIVE OR NEGATIVE IN, A GRADIENT OF THE SALT IN, DIFFERENT KINDS OF WATER							
	Neutral tap water		Distilled water faintly acid		Moderately acid tap water		Strongly acid tap water	
	+	−	+	−	+	−	+	−
Chlorides								
Ammonium	−	−	−	−	21	79	−	−
Potassium	−	−	−	−	67	33	−	−
Sodium	63	37	65	35	76	24	92	8
Calcium	−	−	−	−	26	74	−	−
Magnesium	−	−	−	−	21*	79*	−	−
Nitrates								
Ammonium	−	−	−	−	35	65	−	−
Potassium	30	70	−	−	69	31	−	−
Sodium	−	−	14	86	30	70	80	20
Calcium	50	50	21	79	20	80	−	−
Magnesium	−	−	−	−	0*	100*	−	−
Sulphates								
Ammonium	−	−	−	−	30	70	−	−
Potassium	−	−	−	−	0	100	−	−
Sodium	−	−	−	−	0	100	70	30
Calcium	−	−	−	−	15	85	−	−
Magnesium	−	−	−	−	14*	86*	−	−

* Work done at Chicago.

TABLE 2

Comparing the reactions of fishes to single salts and to combinations of antagonistic salts. One salt is present in .01N concentration and the other as a trace (.0002N). Slightly acid tap contains 6–8 cc. CO_2 per liter; strongly acid 18 cc. CO_2 per liter; distilled water is fainty acid (2–3 cc. CO_2 per liter)

SALT	KIND OF WATER USED IN GRADIENT	REACTION OF FISHES IN PER CENT OF TIME SPENT IN HALVES OF TANK. POSITIVE = IN SALT HALF; NEGATIVE = IN TAP OR DISTILLED WATER HALF	
		Per cent positive	Per cent negative
$NaNO_3$ alone	Slightly acid tap	30	70
$NaNO_3$ + trace $Ca(NO_3)_2$	Slightly acid tap	79	21
$NaNO_3$ + trace $Ca(NO_3)_2$	Strongly acid tap	96	4
$Ca(NO_3)_2$ alone	Slightly acid tap	23	77
$Ca(NO_3)_2$ + trace $NaNO_3$	Slightly acid tap	76	14
$Ca(NO_3)_2$ alone	Distilled water	19	81
$Ca(NO_3)_2$ + trace $NaNO_3$	Distilled water	87	13
$Ca(NO_3)_2$ + trace $Mg(NO_3)_2$	Strongly acid tap	53	47

binations of salts in a gradient, and I present a number of experiments of this sort here. They show that fishes recognize and react to combinations of salts according to the prediction which might have been made from the results of previous work upon antagonism.

It will be remembered that fishes are slightly positive to 0.01N $NaNO_3$ in moderately acid water. It was found, however, that they are negative to this salt in water that is but faintly acid and for this reason the following experiments were run in water which contained less than 8 cc. CO_2 per liter. The experiments were first run as regular salt experiments such as have been described before, and then the antagonizing salt was added to the salt flow. The concentration of the original salt was always 0.01N and that of the antagonizing salt 0.0002N, i.e., a bare trace was added. The results of these experiments are shown in table 2. A considerable number of experiments was run with each combination in the tap water, and then some check experiments were run in distilled water. The results in the distilled water gradients are very similar to those in the tap water.

Discussion of the experiments with antagonizing salt combinations

Table 2 shows clearly that the antagonistic action of the salts is detected and reacted to by the fishes. This is shown, for instance, in the sodium nitrate experiments; here the fishes were 70 per cent negative to this salt in slightly acid water but when a trace of calcium nitrate was added the negative response fell off to 21 per cent and the positive rose from 30 to 79 per cent. Then in strongly acid water the positive response increased to 96 per cent. The reactions of fishes in any gradient are due to their tendency to move about until they reach an environment that neither over- nor under-stimulates them. Thus they will not remain quietly in water that is strongly acid nor will they do so in water that is neutral. A slight degree of acidity (1–6 cc. CO_2 per liter, Wells '15 a) furnishes their optimum stimulation as far as H ion is concerned. The reversal in reaction of the fishes in gradients to which a trace of an antagonistic salt

has been added, must then be due to the fact that this trace of salt lessens the stimulation in the salt end of the gradient. There are three principal factors affecting the degree of stimulation of the gradients referred to in table 2, namely, the original salt (e.g., $NaNO_3$) the antagonising salt (e.g., $Ca(NO_3)_2$, and the acid. Before the antagonising salt was added the fishes were negative to the original salt, even though this meant spending most of the time in a degree of acidity which was slightly above their optimum. With the addition of the antagonising salt, however, they reversed their reaction and became positive to the salt end. The antagonising salt must have diminished the original stimulation in the salt end or have increased the stimulation in the acid end, or both. The work upon the effect of acids and salts upon permeability suggests that both factors were concerned. Lillie ('10) has shown that calcium salts decrease the permeability of egg membranes while the salts of sodium increase the permeability. Osterhout ('12, a and b) has shown that sodium salts increase the permeability of plant cells while the addition of a trace of calcium salt maintains normal permeability even in the presence of an excess of the sodium salt. Osterhout has also shown that there exists a mutual antagonism between certain acids and salts as for instance between HCl and NaCl, but the salts of calcium and magnesium work with rather than against the acid.

In the above experiment, therefore, the addition of the calcium salt to the end of the gradient which contained a sodium salt in concentration strong enough to cause the fishes to give a negative reaction, resulted in the fishes becoming positive. This reversal in the reaction of the fishes must have been due to the decrease in the stimulating power of the salt end. It has already been shown that increasing the acidity of water will cause fishes to become positive to concentrations of sodium salts to which they are normally negative (table 2) and it was found that the higher the acidity the higher was the concentration of sodium salt selected by the fishes.

Table 2 also shows that the antagonistic action between calcium and sodium salts is detected and reacted to, when the con-

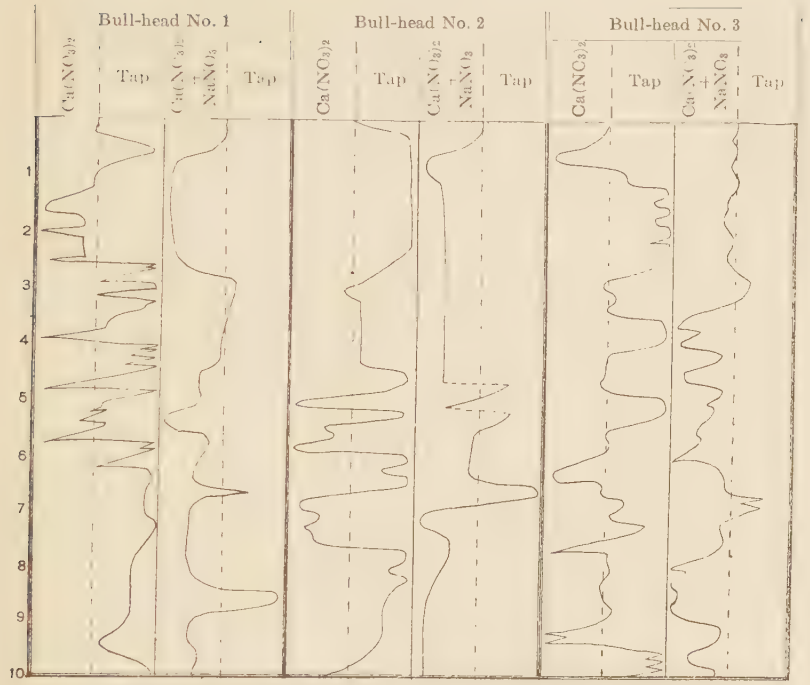

Fig. 2 Showing the reaction of bull-heads (Ameiurus melas) to $Ca(No_3)_2$, alone, and in combination with a trace of $NaNO_3$. The concentration of the calcum salt was 0.01 N throughout and that of the sodium salt 0.0002 N; experiments performed in distilled water; numbers at left indicate time in minutes.

centrations of the two salts are reversed, i.e., when the calcium nitrate is present in 0.01N concentration and the sodium as a trace (0.0002N). The data for table 2 were obtained from graph experiments and also from readings. The same fishes were used in the gradient with the different conditions, i.e., they were first graphed in the gradient with the sodium salt (e.g.) alone and then again after the calcium salt had been added to the flow. To illustrate more accurately this method of experiment figure 2 is inserted. The graphs shown in this figure are 3 of those made by 4 bull-heads. The experiments were run as follows: The gradient with only $Ca(NO_3)_2$ flowing in at the salt end, was obtained by allowing the flow to continue for 30

minutes. The fishes were then taken from the large aquarium and placed in pans of water, numbered 1, etc. The fish from pan No. 1 was placed in the gradient and its movements graphed for 15 minutes. It was removed and No. 2 was placed in the gradient and graphed. This was repeated for Nos. 3 and 4. A trace of $NaNO_3$ was now added to the inflow at the salt end; after 20 minutes fish No. 1 was again placed in the gradient and its movements graphed for 15 minutes. This was repeated for the three remaining fishes in the same order as before. The graphs show the marked difference in the reactions of the fishes before and after the trace of sodium nitrate was added.

C. PHYSIOLOGICAL STATES AND THE REACTIONS OF FISHES

In the discussion so far attention has been called to the fact that in most of the series of experiments, there was a small percentage of the fishes (usually 3–5 per cent) which gave reactions more or less the reverse of those given by the majority. Such exceptions to the general behavior are common in experimental work of all sorts and probably indicate physiological differences upon the part of the organisms. That such physiological differences, i.e., physiological states, exist and that they influence very markedly the reactions of the animals has been proven beyond doubt (Child '13, and Allee '12). Allee and Tashiro ('14) have shown that the reactions of isopods are very closely correlated with the metabolic activity and Allee ('12) has shown that by changing the rate of metabolism he can alter and even reverse the reaction of isopods to current. A correlation between the rate of metabolism and the reactions of amphipods has been shown by Phipps ('15).

At Chicago during the winter of 1913–1914, a study not yet published was being made of the effect of starvation upon the resistance of fishes to KCN and low oxygen; it was thought that the starving fishes furnished good material for ascertaining during the same period something of the effects of starvation upon the reactions of fishes in gradients. Accordingly a series of 89 experiments was run with the starving fishes in gradients; 50

of the experiments were in gradients of $CaCl_2$ since it seemed best to confine the experiments to a few salts at the most. It was decided that the starving fishes should not be handled to any great extent during the obtaining of the data for which the material was originally intended. A few experiments were run in gradients of $Ca(NO_3)_2$ and $MgCl_2$ the results of which were much like those for $CaCl_2$. Nine expriments with starving fishes in low oxygen gradients are included as they are significant.

The experiments with starvation and resistance of fishes showed in brief the following points: The fishes as they began to starve became more resistant to KCN and low oxygen. This rise in resistance which is a decrease in susceptibility, continued for some weeks (varied with species). There was then a rather sudden decrease in resistance (increase in suseptibility) which was found to be a close fore-runner of death. In terms of metabolism, as starvation in certain fishes proceeds the rate of metabolic acitvity is at first decreased. After remaining below normal for some weeks (or even months) the forces which are inhibiting the rate of reaction, give way and the rate runs up rapidly to, and beyond, the normal rate. Whether the changes in the physiological condition of the fishes are wholly quantitative is not certain. It is very probable that a change in the *rate* of metabolism does not express all that takes place but there may be alterations in the kind of metabolism also; in other words starvation in fishes may produce qualitative as well as quantitative changes in metabolism.

Starvation experiments were run with several species of fishes including the rock bass (Ambloplites rupestris), small mouth black bass (Micropterus dolomieu), pumpkin seed (Eupomotus gibbosus), mud minnow (Umbra limi), and the black bull-head (Ameiurus melas). The fishes seemed to be divided into two groups as far as their starvation reactions are concerned. The bull-heads made up one group and the other fishes a second. Most of the work was done with the bull-heads and the rock bass as representatives of the two groups. In the case of the rock bass some quantitative data can be presented.

1. *Reactions of starved fishes to $CaCl_2$*

Normal bull-heads are negative to 0.01N calcium chloride in a gradient. It was noticed, however, that when food was given these fishes they often became positive to the salt half of the tank. To check this reaction 23 experiments with normal, well fed and starved bull-heads were run. Table 3 shows the results obtained. It shows that normal fishes (bull-heads) are negative to 0.01N calcium chloride well-fed ones positive, and starved negative again. The well-fed bull-heads were in fact given all the food they would eat and thus were really over fed, as they ate until their abdomens were much puffed out. The data in

TABLE 3

Showing the reactions of normal, over-fed, and starved bull-heads (Ameiurus melas) to .01N calcium chloride, in a gradient. Data shows per cent of time spent in the halves of the tank

FISH NUMBER	NORMAL REACTION		OVER-FED REACTION		STARVED REACTION	
	$CaCl_2$	Tap	$CaCl_2$	Tap	$CaCl_2$	Tap
1	29	71	66.5	33.5	32	68
2	44	56	78	22	63	37
3	34	66	57	43	29	71
4	37	63	73	27	40	60
5	31	69	78	22	52	48

table 3 is taken from the graphs made with 5 fishes. The normal reaction of each fish was determined immediately upon bringing it into the laboratory from the streams. On the next day the fishes were fed all the beef they would eat and graphed again on the third day. They were then starved and graphed from day to day. The figures in column 4, table 2, are those obtained after from 5 to 10 days starving. Each day calcium chloride was run into the end of the tank opposite that of the day before.

The method of experimenting with the rock bass in the resistance experiments was to bring them in from the creeks in which they live and to weigh them individually, and at once. The process of starvation was then kept track of by successive weighings. Twenty-six experiments with these starving fishes were

run to determine the effect of the starvation upon the reaction to $CaCl_2$. It will be recalled that normal rock bass are negative to this salt in 0.01N concentration (except in the case of large fishes). The starving rock bass were therefore experimented upon in gradients of $CaCl_2$ at different stages of starvation, with results such as those shown in table 4. This table shows that starvation increases the percent of positiveness of these fishes. This is true for that period of starvation, during which the rate of metabolism is slowed up. The few experiments that were performed upon fishes in which the factors inhibiting starvation had broken down and the rate of metabolism had gone above normal, indicate that the fishes are again negative to Ca salts at this time.

TABLE 4

Showing the reactions of normal and starved rock bass (Ambloplites rupestris) to .01N concentrations of $CaCl_2$ in a gradient. Reactions are shown in per cent of time spent in the two halves of the gradient tank

FISH NUMBER	DATE OF COLLECTION	DATE OF EXPERIMENT	ORIGINAL WEIGHT IN GRAMS	WEIGHT AT TIME OF EXPERIMENT	REACTION IN PER CENT OF TIME IN	
					$CaCl_2$	Tap water
	1913	1913				
1	Nov. 20	Nov. 23	9.9	8.9	30	70
2	20	23	23.1	22.5	43	57
3	20	23	56.2	54.5	38	62
4	20	23	70.6	68.4	22	78
5	20	23	126.0	124.0	90	10
6	Oct. 16	23	21.1	18.6	100	0
7	16	23	66.0	61.7	30	70
8	16	23	90.9	77.0	10	90
		1914				
9	Dec. 6	April 9	97.0	64.2	38	62
9	6	10	97.0	64.0	73	27
9	6	10	ends of gradient reversed		82	18
9	6	15	97.0	67.3	34	66
9	6	15	ends of gradient reversed		30	70
9	6	16	97.0	65.0	67	33
9	6	17	97.0	62.3	76	24
10	6	5	83	64.2	46	54
10	6	10	83	61.7	61	39
10	6	10	ends of gradient reversed		58	42
10	6	16	83	61.0	60	40

Note (table 4) that the normal fishes were negative to 0.01N $CaCl_2$, that with the small fishes this reaction had become positive by the end of a little over a month (fish No. 6) while the larger fishes were still negative. Fishes Nos. 9 and 10 show the reaction of fishes starved for almost four months. These fishes were kept in running water and probably obtained a little food but the successive weighings showed that the process of starvation was a continuous one. Note the reversal in reaction of fish No. 9. The first experiment with this fish shows it to be slightly negative. On the next day it had become positive, as was shown by two experiments, with the salt flow at one end of the gradient tank in one, and reversed in the other. The weighings show that the fish had increased in weight since the day before and this increase must have been due to the securing of food in some way; the food had temporarily restored the normal reaction. However, by the next day the weight had again fallen off and the fish was once more positive to the salt, as is characteristic for starving fishes.

2. Reaction of starved fishes to low oxygen

The results of the experiments with starved fishes (rock bass) in low oxygen gradients are seen in table 5, which shows that

TABLE 5

Showing the reactions of normal and starved rock bass to low oxygen in a gradient. Reactions are expressed in per cent of time in the halves of the tank (work done at Chicago)

FISH NUMBER	DATE OF COLLECTION	DATE OF EXPERIMENT	ORIGINAL WEIGHT	WT. AT AT TIME OF EXPT.	REACTIONS IN PER CENT OF TIME IN	
					Low O_2	Tap water
Normal fishes	1913	1913				
1	Nov. 20	Nov. 22	1.7	1.5	5	95
2	20	22	9.9	9.5	25	75
3	20	22	23.1	22.5	32	68
4	20	22	70.6	69.0	20	80
5	20	22	126.0	124.0	91	9
Starved fishes						
6	Oct. 16	22	21.1	18.6	44	56
7	16	22	66.0	61.7	50	50
8	16	22	90.9	77.0	34	66

normal rock bass are negative to low oxygen (1 cc. per liter at the low end) as has been shown also by Shelford and Allee ('13, p. 236). Large rock bass seem to be an exception to this general rule as they are not always negative to low oxygen, and in some cases seem to definitely prefer the low oxygen end of the gradient, spending a majority of the time there. The cause of this reaction has not been determined but it may have to do with the concentration of hydrogen ion which would probably be a little higher in the low oxygen end than in the high oxygen water, the difference being due to the difference in the effect of the two kinds of water upon the elimination of carbon dioxide by the organism.

Fishes Nos. 6 to 8 (table 5) are the individuals occurring under the same numbers in table 4. There it was noted that their reaction to the $CaCl_2$ had become more positive than the normal reaction and in table 5 it will be noted that these fishes are less sensitive to the low oxygen also. Fish No. 5 is also the same in tables 4 and 5 and it will be noted that this fish was positive to both low oxygen and 0.01N $CaCl_2$. Experiments in low oxygen gradients were not performed with these fishes later in their period of starvation but the data given indicate that as they become somewhat starved they at the same time become less negative to low oxygen. This indicates that their metabolic rate is slower than normal.

D. ACCLIMATIZATION AND THE REACTION OF FISHES

During the course of the experiments, considerable evidence was accumulated concerning the effect of acclimatization upon the reactions of the fishes. A few experiments with fishes in CO_2 gradients indicated that these fishes after living for two to three weeks in water whose CO_2 concentration was 8 to 10 cc. per liter, were more sensitive to the CO_2 than normal fishes. To determine whether or not the presence of an excess of salt would result in similar reactions to the salt in a gradient, a series of acclimatization experiments with $CaCl_2$ was run.

A medium-sized (45-gram) rock bass was graphed in a $CaCl_2$ gradient; its normal reaction was decidedly negative to 0.01N

$CaCl_2$. It was now placed in a 20-gallon-jar full of a 0.01N solution of this salt. Each succeeding day it was taken from the jar and its reaction in the gradient graphed, when it was returned to the jar. This was continued for 6 days; the concentration of the solution in the jar was then riased to 0.05N. The fish was left in this solution 4 days longer, being graphed each day. It was then returned to the tap water and graphed again after 2 days. In making the graphs each day, the salt solution was run into the end of the gradient tank, opposite that of the day before. A series of the graphs made by this fish are shown in figure 3. They show the different stages in the process of acclimatization. In short they indicate that the

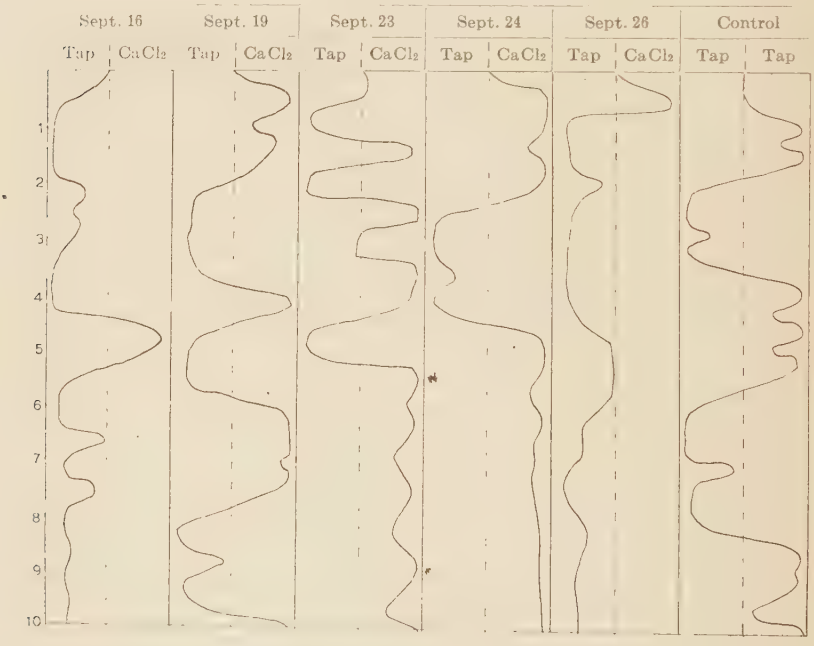

Fig. 3 Showing the reversal in reaction to 0.01 N $CaCl_2$, upon the part of a 45-gram rock bass (Ambloplites rupestris), after being kept in the salt solution for a week, and the return to normal reaction upon being placed in tap water again. In the experiments the salt was made to flow into alternate ends, but in the chart the graphs have been copied so that the reaction will be more easily seen, by keeping the same relation between the tap and $CaCl_2$ ends.

fish did become acclimated to the $CaCl_2$ solution by the end of a week and selected the higher concentration in the gradient. Then after 2 days in the tap water it was negative to the salt again.

A like set of experiments was performed with a small bullhead (6 in. long) with similar results; the acclimatization, however, came sooner. Neither of the fishes was fed while in the $CaCl_2$ and this would have an effect upon their reaction. The fact that the rock bass became negative again after being returned to the tap water indicates that the starvation did not account for its positive reaction while being kept in the $CaCl_2$ solution. Starvation would tend to increase the negativeness of bullheads to the salt so the positive reaction upon being kept in the $CaCl_2$ can be due to nothing but acclimatization. The difference in the effect of starvation upon the reactions of the two species of fishes to salts is probably due to a difference in the metabolism of the fishes and will be discussed in another paper.

E. RESISTANCE OF FISHES TO SALTS

The toxic effect of certain salts upon organisms has been the subject for considerable investigation upon the part of other workers (Ringer, Loeb, R. Lillie, and others) and therefore considerable is known concerning the relative toxicity of the various salt ions. In the present paper are presented data which indicate that much of the work upon the toxicity of salts must be reconsidered and correlated with the chemical reaction of the water. The data show that the poisonous properties of a given salt may vary within wide limits depending upon the amounts of hydrogen or hydroxyl ions present in the solution.

1. Resistance to ammonium salts

According to Mathews ('07) the pharmacological action of most salts is due to the ions of the salt. The kind of action depends upon the character of the charge of the ion, i.e., whether positive or negative; the degree of action is proportional to the available energy in the ion. Ammonia salts are peculiar, how-

ever, in that their toxicity is not due to the action of either of the original ions, but to the products which are derived from the breaking down of the original ammonia compound. Ammonia salts in solution dissociate principally into NH_4 ions and the acid ion with which the ammonia is combined. There is a hydrolytic dissociation also, so that there is always present in the solution a small amount of the free acid and the ammonium hydrate. In considering the reactions of fishes to ammonium hydrate (Wells, '15a, p. 236) it was pointed out that the ammonium hydrate in solution is in equilibrium with and is but a small per cent of the dissolved ammonia gas. In the case of an ammonia salt the hydrolytic dissociation of the salt produces the hydrate, which in turn dissociates to give water and ammonia gas. The amount to which the salts dissociates into ammonium hydrate and ammonia gas varies with the salt, being least in the sulphate and larger in the carbonate (Mathews, l.c.). Mathews further states that it is probable that the action of the ammonium salts is due, therefore, to the hydrate which is formed, and in turn the action of the hydrate is dependent upon the action of the dissociated NH_3. This gas is probably in a nascent condition just at its moment of origin, when the valencies of the nitrogen are still open.

The toxic action of the ammonium salts used in the reaction experiments was found to be very marked when they were dissolved in the tap water, but was much less when the salts were dissolved in distilled water. Solutions (0.01N) of the chloride, nitrate and sulphate were made up in tap and distilled water and small blue-gills (3–gram) were placed in jars of the different solutions. The temperature was kept constant by setting the jars in running tap water. One liter of solution was contained in each; the results are shown in table 6.

The marked increase in the longevity of the fishes in the distilled water seemed worthy of further investigation. Death in the distilled water was in part due to increasing acidity of the solution, as titrations showed a concentration of hydrogen ion at the end of the experiments that must soon have killed the fishes even though no other factor were present. This increase

TABLE 6

Showing the resistance of small blue-gills (3-gram) to .01N concentrations of the chlolide, nitrate and sulphate of ammonium dissolved in tap and distilled water

KIND OF WATER	DYING TIME IN THE SOLUTIONS		
	Chloride	Nitrate	Sulphate
Tap water from aquarium.....	4.8 hours	3.9 hours	3.5 hours
Distilled water.....	18 days	16 days	17 days

in the acidity of the solutions was marked in the case of all three salts and the titrations showed that the acidity upon the day of death of the fishes, had increased to nearly 0.001N while 0.0001N is enough to kill these fishes in distilled water when no salt is present. The increase in acidity was not due entirely to the CO_2 given off by the fishes, as boiling did not remove it. It must, therefore, have come from the acid which had formed from the hydrolysis of the salt. The ammonia formed in the same process had passed off into the atmosphere. It seems clear then that the three salts in question do not furnish a large enough quantity of NH_3 to kill the fishes, if the salts are dissolved in distilled water.

It has been pointed out in a previous paper (Wells '15 a) that the tap water at the University of Illinois contains an unusually large amount of the bicarbonates of Ca and Mg and that as the water is aerated these bicarbonates dissociate to give the normal carbonate. It was thought that the toxicity of the ammonium salts in the tap water may have been due to the formation of ammonium carbonate and the further dissociation of this salt to give NH_3 in toxic quantities. To test this possibility three experiments with 0.01N concentrations of $(NH_4)_2CO_3$ in distilled water, were tried. The dry salt gave a strong odor of ammonia but the solution was too dilute to give any odor at all. After thoroughly shaking the solution and allowing it to stand for 10 minutes, a 10-gram sun-fish was placed in a liter of it. A control was run in distilled water. The result of this experiment, together with those obtained from a number of other experiments are given in table 7. The table shows that am-

monium carbonate is very toxic in distilled water, that standing does not lessen its toxicity greatly nor does it that of the sulphate in tap water, and finally that the ammonium sulphate is no longer toxic in tap water when the carbonates have been converted into sulphates by the addition of enough sulphuric acid to make the water neutral to methyl orange.

TABLE 7

Showing the resistance of fishes to .01N concentrations of $(NH_4)_2CO_3$ in distilled water; the effect of standing upon the toxicity of ammonium salt solutions; and the non toxicity of a solution of $(NH_4)_2SO_4$ in tap water when the carbonates have been converted into sulphates.

SOLUTION	DYING TIME OF THE FISH IN THE	
	Experiment	Control
0.01N $(NH_4)_2CO_3$ in distilled H_2O	1.7 hours	normal
Same after standing 24 hrs	2.2 hours	normal
0.01N $(NH_4)_2SO_4$ in tap water	1.3 hours	normal
Similar solution after 24 hrs	2.2 hours	normal
0.01N $(NH_4)_2SO_4$ in tap water after the carbonates have been changed to sulphates	fish normal at end of a month	control in .01N sulphate in ordinary tap; dead in 2.1 hrs.

The experiments upon the resistance of fishes to ammonium salts show clearly that ammonia in any form is toxic to fishes in water containing carbonates. Since practically all natural waters contain a greater or lesser amount of the carbonates in solution as such, or as bicarbonates, the introduction of even very small amounts of ammonia into these waters will be very detrimental to the fishes. Table 7 shows, on the other hand, that the carbonates are not necessary to the immediate existence of the fishes, i.e., the water need not be alkaline to methyl orange as Marsh ('07) claimed. It may of course be that the carbonates are necessary to a successful completion of the life history of some fishes, or to the continued existence of certain species. This point has not been worked out so far as I am aware.

2. Resistance to potassium salts

Solutions (0.01N) of the chloride, nitrate, and sulphate, were made up in tap water and a small blue gill (3–5 gram) introduced into a liter of each; the results are shown in table 8.

The action of the potassium salts in tap water was checked by placing a fish in a 0.01N solution of the most toxic one, i.e., the sulphate, in distilled water. The reactions of this fish were very peculiar. After 3 days in the solution it was noticed that the fish was losing its equilibrium and it was expected that it would die in a few hours. On the next day, however, it was still

TABLE 8

Showing the resistance of small blue gills to .01N concentrations of potassium salts in solution in tap water

	DYING TIME IN		
	Chloride	Nitrate	Sulphate
Normal on 15th day.....		15 days	4 days

alive and for 10 days more it lived spending much of the time lying on its side but righting itself when touched with a glass rod. Its movements were sluggish and stiff, much as though it were dying from fungus disease. In all, the fish lived for 14 days in 0.01N potassium sulphate solution, which is over three times as long as a fish of the same size lived in the same strength solution in tap water. The long-drawn-out death of the fish is not a phenomenon that is peculiar to potassium salts, however, for it was noted that another small blue gill which was in an ammonium nitrate experiment in distilled water at the same time, gave a similar reaction. This latter fish swam about for three days on its side with the body bent into the bow-shape that often distorts fish after death, especially when they dry out. This suggests that the distortion may have been due to osmotic changes in the tissues.

3. Resistance to sodium salts

Experiments with the following sodium salts were performed in tap water: bicarbonate, carbonate, chloride, nitrate, and sulphate. The solutions were 0.01N and the fishes small blue gills (3–5 grams). The results were as follows:

Salt used	*Resistance of fishes*
Sodium bicarbonate	Normal at end of 15 days; discont.
Sodium carbonate	Dead after 3 days
Sodium chloride	Normal after 19 days; discont.
Sodium nitrate	Dead after 31 days; only 50 cc. water left
Sodium sulphate	Normal after 20 days; discont.

From the above results we see that the sodium salts are not toxic to blue gills when 0.01N concentrations are used in tap water. The carbonate is an exception as the fish dies in this solution in 3 days. It has already been shown (Wells '15 a) that these fishes cannot live in water that is even faintly alkaline and thus the action of the carbonate is due to its alkalinity.

It will be remembered that the reactions of the fishes in salt gradients were complicated by the antagonism between the salts and the acid in the water. Loeb was the first to demonstrate that there exists an antagonism between salts and acids, as in 1899 he showed that acid antagonises the effect of NaCl on the swelling of muscle. He suggested that the antagonism depends upon the action of the substances upon the proteins of the tissues. Again, Loeb and Wasteneys ('11 and '12) demonstrated the antagonism between salts and acids, in their effect upon the marine fish Fundulus and explained the effect as due to a direct action on permeability. Osterhout ('14) made investigations which show that similar though less striking antagonism between acids and NaCl occurs in plants; he further states that the antagonism is not as great as that between NaCl and $CaCl_2$.

To determine the relation of the antagonism between salts and acids to the resistance of fresh water fishes, a series of experiments was run with NaCl and HCl. Table 9 summarizes the results of these experiments. From this table it will be noted that fresh water fishes of the same species and size live much longer in toxic

TABLE 9

Showing the antagonism of NaCl and HCl in their toxic action upon fishes; experiments performed in distilled water (U. of I.)

SIZE AND SPECIES OF FISH	KIND OF SOLUTION	DYING TIME IN HOURS
25-gram rock bass	0.25N NaCl	18
23-gram rock bass	0.25N NaCl + 0.00005N HCl	41
12- gram green spotted sun-fish	0.25N NaCl	48
8- gram green spotted sun-fish	0.25N NaCl + 0.00005N HCl	144
3-gram green spotted sun-fish	0.0001N HCl	48
3-gram green spotted sun-fish	0.0001N HCl + 0.12N NaCl	normal at end of month
45-gram green spotted sun-fish	0.25N NaCl + KOH to make just alk.	14

concentrations of NaCl when a trace of HCl is added. Also that fishes in toxic concentrations of HCl live longer when NaCl is present. Furthermore, NaCl is much more toxic in faintly alkaline solutions than it is in faintly acid solutions. All this agrees with Osterhout's conclusions as to the effect of alkalies and acids on permeability.

4. Resistance to the salts of Ca and Mg

The only resistance experiments which have been carried on with these salts are some that were performed at Chicago. The experiments with Ca were performed in connection with the acclimatization experiments already discussed. In brief, it was found that the sun-fishes lived very well in 0.01N $CaCl_2$, while the bull-heads did not live so well. Other experiments showed this same relation for the nitrate and sulphate but the latter salts were decidedly more toxic than the chloride and the sun-fishes did not live well in solutions of them. An interesting fact was noted in connection with the $CaCl_2$ experiments. A medium sized (50-gram) rock bass, after a week in 0.01N solution, showed signs of degeneration of the rays of the tail fin. This degeneration continued until nothing but the blood-reddened stub of the tail was left. The other fins were not affected; the tail fin regenerated when the fish was returned to tap water.

Day ('87, p. 203) states that in a certain lake in the British Isles, there is a race of tailless trout which some authors claim can be traced as due to the action of deleterious matter in the water. Day (loc. cit) also quotes J. Harvie-Brown as saying, about 1876, "that a contraction of the rays of the tail fins of the trout in the river Carron occurred, and was believed to be due to the continuous pollution of the water through the agency of paper mills." Upon looking up the composition of the waste from the paper mills (Griffin and Little '94, and Phelps '09) I find that among other substances calcium is always present in large quantities, both as the chloride and in other combinations. Therefore the phenomenon reported by Day was likely due to the presence of an excess of calcium in the water.

Marsh ('07) has shown that the waste from paper mills is very toxic to fishes. Calcium is not especially important, however, as the toxicity of the waste is probably due to the excess of acidity or alkalinity, and perhaps to other toxic substances.

V. GENERAL DISCUSSION

The experiments discussed in the preceding pages will be considered very briefly in one or two phases of their general bearing. From an ecological point of view they emphasize once more the ability of fishes to recognize and react to environmental factors in very small concentration. It should be pointed out, however, that the reactions of fishes to salts in solution are by no means so delicate as their reactions to acids and alkalies, i.e., to hydrogen and hydroxyl ions. As a matter of fact the reaction to salts is complicated by the acid factor in many cases, as, for instance, when the salt gives an acid solution, but more especially in the numerous instances where there exists an antagonism between the salt and the acid. Thus fishes may react differently to a given salt concentration in water which is strongly acid and water that is but faintly acid. The resistance experiments show, also, that fishes can live in the presence of an acid concentration which would ordinarily kill them, if the proper concentration of the right salts is present. The work of Osterhout ('15) and others, as well as data presented in this paper,

indicates that the antagonism between the salts of calcium and magnesium is not nearly so marked as it is in the case of the salts of sodium and potassium. Since the former salts are by far the most common and plentiful in natural fresh waters, the importance of salts in nature in antagonising introduced acids is less than it would be were the salts of sodium and potassium plentiful. The problem is one which will furnish material for some very interesting ecological investigation.

The importance of small amounts of ammonia in natural waters has been pointed out in the discussion of these salts. The effects of starvation upon fish metabolism and reaction will be further discussed in another paper. There is an interesting possibility brought out by the acclimatization and other data, especially those pertaining to the importance of acids, that will be discussed here. This possibility relates to the movements of organisms in general but the present discussion will be limited to the very interesting migrations of the anadromous fishes.

The stimulus that causes anadromous fishes to spend part of their life cycle in fresh and part in salt water has long been a matter for speculation. Such stimulus must be related to the rhythmical metabolism of the animal, for it brings the fishes into the sea or fresh water at certain definite stages in the life cycle. The state of the metabolism of these fishes while they are in the fresh water, must differ very decidedly from that during the period of the life cycle which they spend in the ocean, for these two environments differ in two very important particulars, namely, the fresh water has a low specific gravity and is consistently acid in reaction, while the sea water has a relatively high specific gravity and is consistently alkaline. Also the reactions of the fishes are markedly different. In the fresh water they are positive to current, and, in a gradient, select water that is just on the acid side of neutrality and of lower density than that of the sea. Salt water fishes, on the other hand, are probably negative to a fresh water current, select water on the alkaline side of neutrality and reject water of low specific gravity for that of higher (Shelford and Powers '15). The reactions of the fishes in fresh water,

therefore, are the reverse of those in sea-water with regard to these three factors, and in the normal life cycle of such anadromous fishes as the salmon, this reversal in reaction must occur at least twice, once when the fishes leave the fresh water streams for the ocean, and again when they return. With species of salmon that breed more than once, the reversal must occur more often.

There are two general complexes of factors to be considered in an attempted explanation of the reactions of the anadromous fishes, namely, the fish and the environment. Both are made up of physico-chemical factors which are measurable, and to a large degree quantitatively. Of the two complexes, that of the living organism is least understood and perhaps, because it is much more variable and changing than the environmental complex, which, especially in the case of the sea-water, varies hardly at all. For the fishes to live normally in the environment there must exist between the two complexes a more or less complete equilibrium. A disturbance of this equilibrium resulting from a change in either of the complexes, will, if great enough or long enough continued, result in the death of the fishes unless by their reactions they seek out another environment which allows their physiological processes to proceed normally. It should be emphasized that the only mode of readjustment is through the proper reaction, either physiological or motile upon the part of the fishes, since the environment is much the more stable complex, and there is a great deal of evidence to show that of the two possible reactions upon the part of the living organism, the motile reaction is much more likely to occur than the physiological readjustment, i.e., acclimatization. The data presented in this paper and the one preceding (Wells '15a) as well as that by Shelford and Allee ('13) and Shelford and Powers ('15) show that fishes will react to environmental factors in a way that will tend to remove them from detrimental conditions, long before the adjustment becomes a matter of life and death. Thus we find the salmon leaving the fresh water for the ocean, when, as will be pointed out later, it has been shown (Day '87) that remaining in the fresh water for the entire life cycle would not

result fatally either to the individual or to the species. The mechanism, therefore, which is working to preserve the life of the organism is so delicate that it produces beneficial reactions upon the part of the animal far in advance of life and death complications. The working of this mechanism is undoubtedly closely correlated with quantitative and perhaps qualitative changes in metabolism. These changes in metabolism will have a direct relation to the amount of CO_2 given off by the organism.

It has been shown that a slight increase in the carbon dioxide content of an animal's blood results in a marked increase in the general irritability, and this increase in irritability would alone result in an increase in the range and vigor of the movements made by the organism. Thus no factor other than increased metabolism need be hypothecated to account for the stimulus which starts the breeding migration of so many animals. The directive factors which result in the animal's coming into special conditions for the breeding activities are another matter. These can be none other than the factors, physical and chemical, which are present in the environment. In the general metabolism of fishes, the stage of development of the sex organs plays an important rôle, and it is very probable that the state of metabolism in these organs furnishes the initial stimulus which causes the animals to start upon the breeding migrations at a given period of the life cycle. Treadwell ('15) points out that the eggs of the Atlantic palola give off an increasing amount of CO_2 as the swarming season approaches, and concludes that this indicates that there is probably an internal stimulus which is important in producing the swarm. There can be little doubt but that such internal stimulus is acting; the important fact, however, is that it has been shown that such internal changes in the physiological state of the animal may result in very marked changes in the animal's reactions to environmental factors. Allee ('12) has shown that, in isopods, a high rate of metabolism is correlated with a high percent of positive responses to current and that a lowering of the metabolic rate in the animals will diminish and even reverse the rheotactic reaction.

If we consider the different reactions of the salmon to current, acidity, and density, at different stages in the life cycle, beginning with the hatching of the egg we may proceed as follows. It is a well established fact (Loeb '13) that in the fertilized egg and newly hatched fry, the rate of oxidation is high, and it seems to be clear (Wells '13) that from this time on, up to sexual maturity the rate runs down. That is, the rate varies inversely with the age of the fish. Salmon eggs hatched in fresh water must develop into fry which are able to live in slightly acid water, of relatively low density, and the fishes must also be positive to current or they will be swept from the stream. This we find is true and thus ability to live in fresh water is correlated at this time with a high metabolic rate. As time goes by, however, the rate of reaction becomes gradually lower until we find the fishes either becoming actively negative, or at least indifferent to current, and they are swept or swim into the ocean. They now live for some time in the alkaline water of the ocean, and are able to withstand its much higher density. The equilibrium between the environment and the organism is again disturbed after a time, however, and we find the fishes once more selecting the fresh water at another period of high metabolic rate, i.e., with the maturing of the sex glands. From this it would seem entirely possible that fishes which are normally fresh water forms might be temporarily transformed into salt water forms by regulating, that is lowering, the rate of metabolism.

With regard to the selection of the water of greater or lesser density, the data presented in this paper offer an interesting possibility. It has been shown that fresh water fishes whose metabolic rate has been lowered by starvation, will select a notably higher concentration of $CaCl_2$ in the gradient than will normal fishes. Also older fishes select a higher concentration than do younger ones. Thus a lowering of the metabolism causes the fishes to choose a medium with higher specific gravity than that normally chosen. It will be remembered furthermore that a stay of a little less than a week in 0.01N $CaCl_2$ solution caused a fish that was normally negative to this concentration in a gradient, to become positive. Upon being returned to the tap

water the reaction was again reversed and the fish became negative once more.

Acclimatization of fishes to salts must certainly be concerned with internal adjustments, for Sumner ('07) has shown that the specific gravity of fishes' blood is altered when they are changed from fresh to sea-water, and vice versa. An alteration in the density of the blood seems then to result in a reversal in the reactions of the organism to density in the environment. Green ('04) has shown that changes in the specific gravity of the blood of the salmon occur at the time the fishes are entering the fresh water; the blood gradually acquires a density that averages 17.6 per cent less than that of salmon in sea-water (l.c., p. 454). Jones ('87) has proved that age, exercise, sexual maturity, pregnancy, food, etc., have a measurable influence upon the density of the blood of man, and Sumner ('07) states that there are seasonable differences displayed by fishes, in the osmotic phenomena through their gills. It may be that the specific gravity of the blood of anadromous fishes at different stages in the life cycle, can be used as an index to the physiological changes that are going on in the organism. Also the effect upon the organism of a higher CO_2 production within the tissues must vary with the density of the blood and would probably be more marked when the blood is less dense.

An investigation of the changes in the density of the blood of the salmon could perhaps best be begun with the fry in the fresh water streams. As the fishes remain for 2 years or even 3 in the fresh water before leaving for the ocean, a thorough study of the relative densities of the blood and the fresh water could be made in this period. That the instinct which causes these fishes finally to reject the fresh water for that of the sea, is backed by some very strong stimulus is indicated by data given by Day ('87). Day speaks of an experiment which was carried on by Maitland in 1880. Eggs of salmon were hatched in fresh water, and the young salmon were placed in ponds shut off from the sea. These fishes ate well and grew vigorously until they were about $2\frac{1}{2}$ years old. At this stage in the life history, the individuals are known as 'smolts' and it is at the smolt stage that they

leave the fresh water. In October, 1883, one of the fishes jumped out of the pond onto the bank. By the end of November, several had jumped out onto the bank and died there (they usually jumped during the night or early morning). In the following May, 16 of the fishes were found dead on the bank. Then the following October (1884) they commenced constantly jumping out of the pond and meeting with fatal injuries. It was observed that the fishes did not feed at this latter date; this failure to take food is characteristic of salmon entering fresh water to breed.

Examination of the fishes which had jumped out of the pond showed that all were approaching maturity and in the later cases, the eggs and sperm were ripe. An attempt was made to fertilize the eggs with the sperm, with good success. Day states that this second generation was normal and vigorous up to 20 months and concluded that it was definitely proved that a sojourn in salt water is not necessarily for the development of the sexual products. If this is true, the migration of the salmon into the salt water, and back again, is all the more curious. There would be advantages and disadvantages to such behavior but the above data prove that the fishes are reacting to the environment in a way that is not immediately essential though the stimulus seems to be a very strong one. A study of the behavior of these fishes in salt, acid and alkali gradients at different stages in their life history, would undoubtedly prove very suggestive and such a study correlated with physiological investigations of the fishes at similar stages will without doubt solve the question of the movements of anadromous fishes.

VI. GENERAL CONCLUSIONS

1. Fresh water fishes recognize and react to the presence of salts in solution. The reaction is one which tends to bring them into their optimum salt concentration.

2. Fresh water fishes (and probably marine fishes also, Shelford and Powers '15) are not as sensitive to salt ions as they are

to hydrogen and hydroxyl ions. The reactions to either the ions of salts or acids are complicated by the presence of the ions of the other.

3. Fresh water fishes react to combinations of antagonistic salts or to an antagonistic salt and acid, in a way that tends to bring them into a region of optimum stimulation. The phenomena of antagonism are thus indicated by the behavior as well as the resistance of organisms.

4. Starvation causes certain fishes (e.g., Ambloplites rupestris, rock bass) to select higher concentrations of salt than those normally selected. Other fishes (Ameiurus melas, bull-head) when starved, select lower concentrations than normally. Overfeeding causes bull-heads to select higher concentrations, than those normally chosen.

5. Rock bass and bull-heads which are normally negative to $CaCl_2$ 0.01N solution, become positive after being kept in this concentration for about a week. They become negative again when returned to tap water for 24 hours.

6. The migrations of anadromous fishes are probably correlated with rhythmic changes in metabolism. These alterations in metabolic activity are largely the result of internal changes such as occur with the ripening of the sexual products.

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VITA

Morris Miller Wells was born in Kansas in 1885. He received his preparatory training in the Kansas State Normal school where he completed the four year Normal course in 1909. The year of 1909-10 he spent as teacher of science in the Clay County High School at Clay Center, Kanas. In the summer of 1910 he entered the University of Chicago where he remained for the two succeeding years and completed three years of undergraduate work taking the degree of B. S. in June, 1912. During most of his undergraduate work he had a scholarship and at the time of graduation was elected to Phi Beta Kappa. He continued in the University of Chicago during the years 1913 to 1915 including summer terms. The first year of graduate work was done under an assistant's appointment and the second as fellow; his major work was done in Zoology. During the academic year 1912-13 he was elected to Sigma Xi. In the fall of 1914 he went to the University of Illinois where he had accepted a fellowship in the Department of Zoology and where he completed his work for the degree of Doctor of Philosophy in June, 1915. During the period of graduate study he published the following papers:

1. The Resistance of Fishes to Different Concentrations and Combinations of Oxygen and Carbon Dioxide. Biological Bulletin, vol. 25, pp. 323-347, November, 1913.

2. The Reactions and Resistance of Fishes to Temperature, Transactions of the Illinois Academy of Science, vol. 7, 1914.